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(54) Title: CANCER ASSOCIATED ANTIGENS AND USES THEREFOR

(57) Abstract: Cancer associated antigens have been identified by autologous antibody screening of libraries of nucleic acids expressed in testis cells using antisera from seminoma patients. The invention relates to nucleic acids and encoded polypeptides which are cancer associated antigens expressed in patients afflicted with a variety of cancers. The invention provides, *inter alia*, isolated nucleic acid molecules, expression vectors containing those molecules and host cells transfected with those molecules. The invention also provides isolated proteins and peptides, antibodies to those proteins and peptides and cytotoxic T lymphocytes which recognize the proteins and peptides. Fragments of the foregoing including functional fragments and variants also are provided. Kits containing the foregoing molecules additionally are provided. The molecules provided by the invention can be used in the diagnosis, monitoring, research, or treatment of conditions characterized by the expression of one or more cancer associated antigens.

**CANCER ASSOCIATED ANTIGENS AND USES THEREFOR****Field of the Invention**

The invention relates to nucleic acids and encoded polypeptides which are cancer  
5 associated antigens. The invention also relates to agents which bind the nucleic acids or  
polypeptides. The nucleic acid molecules, polypeptides coded for by such molecules and  
peptides derived therefrom, as well as related antibodies and cytolytic T lymphocytes, are  
useful, *inter alia*, in diagnostic and therapeutic contexts.

**Background of the Invention**

10 The mechanism by which T cells recognize foreign materials has been implicated in  
cancer. A number of cytolytic T lymphocyte (CTL) clones directed against autologous  
melanoma antigens, testicular antigens, and melanocyte differentiation antigens have been  
described. In many instances, the antigens recognized by these clones have been  
15 characterized.

The use of autologous CTLs for identifying tumor antigens requires that the target  
cells which express the antigens can be cultured *in vitro* and that stable lines of autologous  
CTL clones which recognize the antigen-expressing cells can be isolated and propagated.  
While this approach has worked well for melanoma antigens, other tumor types, such as  
20 epithelial cancers including breast and colon cancer, have proved refractory to the approach.

More recently a serological cloning approach named SEREX (serological analysis of  
tumor antigens by recombinant cDNA expression cloning) was described by Sahin et al.  
(*Proc. Natl. Acad. Sci. USA* 92:11810-11813, 1995). Also, see U.S. Patent No. 5,698,396,  
incorporated herein by reference. According to this approach, autologous antisera are used to  
25 identify immunogenic protein antigens expressed in cancer cells by screening expression  
libraries constructed from tumor cell cDNA. Antigen-encoding clones so identified have been  
found to have elicited an high-titer humoral immune response in the patients from which the  
antisera were obtained. Such a high-titer IgG response implies helper T cell recognition of the  
detected antigen. These tumor antigens can then be screened for the presence of MHC/HLA  
30 class I and class II motifs and reactivity with CTLs.

Applying SEREX to a range of tumor types, a number of novel cancer-associated  
immunogenic gene products have been cloned (reviewed in Türeci et al., *Mol. Med. Today*,

3:342-349, 1997; Sahin et al., *Curr. Opin. Immunol.*, 9:709-716, 1997; Old et al., *J. Exp. Med.*, 187:1163-1167, 1998), among them tumor-specific antigens which had been originally identified as targets of CTLs, e.g. MAGE and tyrosinase. According to their expression profile, gene/transcript structure, and immunogenicity different groups of tumor antigens can be distinguished. These include differentiation antigens, overexpressed immunogenic proteins, mutated gene products and tumor-specific splice variants (Sahin, et al., 1997).

Among the most interesting is the group of cancer/testis antigens (CT antigens, or CTAs), which is characterized by its intriguing expression pattern. CT antigens are expressed by a variable proportion (ranging from 10% to 70%) of a wide range of different human tumor types (Chen et al., *Proc. Natl. Acad. Sci. USA*, 94:1914-1918, 1997). Cancer/testis antigens are not found in normal tissues other than testis since their respective encoding genes - the so-called cancer/testis genes (CTGs) (Türeci et al., *Proc. Natl. Acad. Sci. USA*, 95:5211-5216, 1998) - are only transcribed in malignant cells. The prototypes of CTAs, MAGE, BAGE, and GAGE, were initially identified as targets for CTLs.

Several new members were added by SEREX to this category (Türeci et al., *Cancer Res.* 56:4766-4772, 1996; Chen et al., 1997; Türeci et al., 1998; Chen et al., *Proc. Natl. Acad. Sci. USA*, 95:6919-6923, 1998). The stringent restriction of their expression pattern makes CTAs ideal candidates for cancer vaccination.

Since individual CTAs are expressed only in a variable proportion of tumors of a given entity, the availability of additional CTAs would significantly enlarge the proportion of patients who are potentially eligible for therapeutic interventions. Despite the fact that the pool of available tumor antigens has grown since the introduction of SEREX, the proportion of antigen-negative tumors is still high, particularly in frequent neoplasms such as colon and prostate cancer (Sahin et al., *Int. J. Cancer*, 78:387-389, 1998). Thus there presently is a need for additional cancer antigens for development of therapeutics and diagnostics applicable to a greater number of cancer patients having various cancers.

### **Summary of the Invention**

Autologous antibody screening has now been applied to a testis-enriched cDNA library using antisera from a seminoma patient. To enhance the detection of CTAs, the original SEREX technique was modified. To this end, testis expression libraries were enriched for testis-specific transcripts by subtractive techniques and immunoscreened with

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allogeneic sera from cancer patients. Several novel cancer associated antigens have been identified. The invention provides, *inter alia*, isolated nucleic acid molecules, expression vectors containing those molecules and host cells transfected with those molecules. The invention also provides isolated proteins and peptides, antibodies to those proteins and peptides and CTLs which recognize the proteins and peptides. Fragments including functional fragments and variants of the foregoing also are provided. Kits containing the foregoing molecules additionally are provided. The foregoing can be used in the diagnosis, monitoring, research, or treatment of conditions characterized by the expression of one or more cancer associated antigens.

Prior to the present invention, only a handful of cancer associated genes had been identified in the past 20 years. The invention involves the surprising discovery of several genes, one previously known and the remainder previously unknown, which are expressed in individuals who have cancer. These individuals all have serum antibodies against the proteins (or fragments thereof) encoded by these genes. Thus, abnormally expressed genes are recognized by the host's immune system and therefore can form a basis for diagnosis, monitoring and therapy.

The invention involves the use of a single material, a plurality of different materials and even large panels and combinations of materials. For example, a single gene, a single protein encoded by a gene, a single functional fragment thereof, a single antibody thereto, etc. can be used in methods and products of the invention. Likewise, pairs, groups and even panels of these materials and optionally other cancer associated antigen genes and/or gene products can be used for diagnosis, monitoring and therapy. The pairs, groups or panels can involve 2, 3, 4, 5 or more genes, gene products, fragments thereof or agents that recognize such materials. A plurality of such materials are not only useful in monitoring, typing, characterizing and diagnosing cells abnormally expressing such genes, but a plurality of such materials can be used therapeutically. An example of this is the use of a plurality of such materials prophylactically or acutely for the prevention, delay of onset, amelioration, etc. of cancer in cells which express or will express such genes. Any and all combinations of the genes, gene products, and materials which recognize the genes and gene products can be tested and identified for use according to the invention. It would be far too lengthy to recite all such combinations; those skilled in the art, particularly in view of the teaching contained herein, will readily be able to determine which combinations are most appropriate for which



circumstances.

As will be clear from the following discussion, the invention has *in vivo* and *in vitro* uses, including for therapeutic, diagnostic, monitoring and research purposes. One aspect of the invention is the ability to fingerprint a cell expressing a number of the genes identified according to the invention by, for example, quantifying the expression of such gene products. Such fingerprints will be characteristic, for example, of the stage of the cancer, the type of the cancer, or even the effect in animal models of a therapy on a cancer. Cells also can be screened to determine whether such cells abnormally express the genes identified according to the invention.

The invention, in one aspect, is a method of diagnosing a disorder characterized by expression of a cancer associated antigen precursor coded for by a nucleic acid molecule. The method involves the steps of contacting a biological sample isolated from a subject with an agent that specifically binds to the nucleic acid molecule, an expression product thereof, or a fragment of an expression product thereof complexed with an MHC, preferably an HLA, molecule, wherein the nucleic acid molecule is a NA Group 1 nucleic acid molecule, and determining the interaction between the agent and the nucleic acid molecule, the expression product or fragment of the expression product as a determination of the disorder.

In one embodiment the agent is selected from the group consisting of (a) a nucleic acid molecule comprising NA Group 1 nucleic acid molecules or a fragment thereof, (b) a nucleic acid molecule comprising NA Group 3 nucleic acid molecules or a fragment thereof, (c) a nucleic acid molecule comprising NA Group 5 nucleic acid molecules or a fragment thereof, (d) an antibody that binds to an expression product, or a fragment thereof, of NA group 1 nucleic acids, (e) an antibody that binds to an expression product, or a fragment thereof, of NA group 3 nucleic acids, (f) an antibody that binds to an expression product, or a fragment thereof, of NA group 5 nucleic acids, (g) an agent that binds to a complex of an MHC, preferably HLA, molecule and a fragment of an expression product of a NA Group 1 nucleic acid, (h) an agent that binds to a complex of an MHC, preferably HLA, molecule and a fragment of an expression product of a NA group 3 nucleic acid, and (i) an agent that binds to a complex of an MHC, preferably HLA, molecule and a fragment of an expression product of a NA Group 5 nucleic acid.

The disorder may be characterized by expression of a plurality of cancer associated antigen precursors. Thus the methods of diagnosis may include use of a plurality of agents,

each of which is specific for a different human cancer associated antigen precursor (including at least one of the cancer associated antigen precursors disclosed herein), and wherein said plurality of agents is at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9 or at least 10 such agents.

5 In each of the above embodiments the agent may be specific for a human cancer associated antigen precursor, including the seminoma cancer associated antigen precursors disclosed herein.

In another aspect the invention is a method for determining regression, progression or onset of a condition characterized by expression of abnormal levels of a protein encoded by a nucleic acid molecule that is a NA Group 1 molecule. The method involves the steps of  
10 monitoring a sample, from a subject who has or is suspected of having the condition, for a parameter selected from the group consisting of (i) the protein, (ii) a peptide derived from the protein, (iii) an antibody which selectively binds the protein or peptide, and (iv) cytolytic T cells specific for a complex of the peptide derived from the protein and an MHC molecule, as  
15 a determination of regression, progression or onset of said condition. In one embodiment the sample is a body fluid, a body effusion or a tissue.

In another embodiment the step of monitoring comprises contacting the sample with a detectable agent selected from the group consisting of (a) an antibody which selectively binds the protein of (i), or the peptide of (ii), (b) a protein or peptide which binds the antibody of  
20 (iii), and (c) a cell which presents the complex of the peptide and MHC molecule of (iv). In a preferred embodiment the antibody, the protein, the peptide or the cell is labeled with a detectable molecule, such as a radioactive label or an enzyme. The sample in a preferred embodiment is assayed for the peptide.

According to another embodiment the nucleic acid molecule is one of the following: a  
25 NA Group 3 molecule or a NA Group 5 molecule. In yet another embodiment the protein is a plurality of proteins, the parameter is a plurality of parameters, each of the plurality of parameters being specific for a different of the plurality of proteins.

The invention in another aspect is a pharmaceutical preparation for a human subject. The pharmaceutical preparation includes an agent which when administered to the subject  
30 enriches selectively the presence of complexes of an HLA molecule and a human cancer associated antigen, and a pharmaceutically acceptable carrier, wherein the human cancer associated antigen is a fragment of a human cancer associated antigen precursor encoded by a

nucleic acid molecule which comprises a NA Group 1 molecule. In one embodiment the nucleic acid molecule is a NA Group 3 nucleic acid molecule.

The agent in one embodiment comprises a plurality of agents, each of which enriches selectively in the subject complexes of an HLA molecule and a different human cancer associated antigen. Preferably the plurality is at least two, at least three, at least four or at  
5 least five different such agents.

In another embodiment the agent is selected from the group consisting of (1) an isolated polypeptide comprising the human cancer associated antigen, or a functional variant thereof, (2) an isolated nucleic acid operably linked to a promoter for expressing the isolated  
10 polypeptide, or functional variant thereof, (3) a host cell expressing the isolated polypeptide, or functional variant thereof, and (4) isolated complexes of the polypeptide, or functional variants thereof, and an HLA molecule.

The agent may be a cell expressing an isolated polypeptide. In one embodiment the agent is a cell expressing an isolated polypeptide comprising the human cancer associated  
15 antigen or a functional variant thereof. In another embodiment the agent is a cell expressing an isolated polypeptide comprising the human cancer associated antigen or a functional variant thereof, and wherein the cell expresses an HLA molecule that binds the polypeptide. The cell can express one or both of the polypeptide and HLA molecule recombinantly. In preferred embodiments the cell is nonproliferative. In yet another embodiment the agent is at  
20 least two, at least three, at least four or at least five different polypeptides, each representing a different human cancer associated antigen or functional variant thereof.

The agent in one embodiment is a PP Group 2 polypeptide. In other embodiments the agent is a PP Group 3 polypeptide or a PP Group 4 polypeptide.

In an embodiment each of the pharmaceutical preparations described herein also  
25 includes an adjuvant.

According to another aspect the invention, a composition is provided which includes an isolated agent that binds selectively a PP Group 1 polypeptide. In separate embodiments the agent binds selectively to a polypeptide selected from the following: a PP Group 2 polypeptide, a PP Group 3 polypeptide, a PP Group 4 polypeptide, and a PP Group 5  
30 polypeptide. In other embodiments, the agent is a plurality of different agents that bind selectively at least two, at least three, at least four, or at least five different such polypeptides.

In each of the above described embodiments the agent may be an antibody.

In another aspect the invention is a composition of matter composed of a conjugate of the agent of the above-described compositions of the invention and a therapeutic or diagnostic agent. Preferably the conjugate is of the agent and a therapeutic or diagnostic that is an antineoplastic.

5           The invention in another aspect is a pharmaceutical composition which includes an isolated nucleic acid molecule selected from the group consisting of: (1) NA Group 1 molecules, and (2) NA Group 2 molecules, and a pharmaceutically acceptable carrier. In one embodiment the isolated nucleic acid molecule comprises a NA Group 3 or NA Group 4 molecule. In another embodiment the isolated nucleic acid molecule comprises at least two  
10 isolated nucleic acid molecules coding for two different polypeptides, each polypeptide comprising a different cancer associated antigen.

          Preferably the pharmaceutical composition also includes an expression vector with a promoter operably linked to the isolated nucleic acid molecule. In another embodiment the pharmaceutical composition also includes a host cell recombinantly expressing the isolated  
15 nucleic acid molecule.

          According to another aspect of the invention a pharmaceutical composition is provided. The pharmaceutical composition includes an isolated polypeptide comprising a PP Group 1 or a PP Group 2 polypeptide, and a pharmaceutically acceptable carrier. In one embodiment the isolated polypeptide comprises a PP Group 3 or a PP Group 4 polypeptide.

20           In another embodiment the isolated polypeptide comprises at least two different polypeptides, each comprising a different cancer associated antigen at least one of which is encoded by a NA group 1 molecule as disclosed herein. In separate embodiments the isolated polypeptides are selected from the following: PP Group 3 polypeptides or HLA binding fragments thereof and PP Group 5 polypeptides or HLA binding fragments thereof.

25           In an embodiment each of the pharmaceutical compositions described herein also includes an adjuvant.

          Another aspect the invention is an isolated nucleic acid molecule comprising a NA Group 3 molecule. Another aspect the invention is an isolated nucleic acid molecule comprising a NA Group 4 molecule.

30           The invention in another aspect is an isolated nucleic acid molecule selected from the group consisting of (a) a fragment of a nucleic acid selected from the group of nucleic acid molecules consisting of SEQ ID Nos numbered below and comprising all nucleic acid

sequences among SEQ ID NOs:5-10, of sufficient length to represent a sequence unique within the human genome, and identifying a nucleic acid encoding a human cancer associated antigen precursor, (b) complements of (a), provided that the fragment includes a sequence of contiguous nucleotides which is not identical to any sequence selected from the sequence group consisting of (1) sequences having the GenBank accession numbers of Table 6, (2) complements of (1), and (3) fragments of (1) and (2).

In one embodiment the sequence of contiguous nucleotides is selected from the group consisting of: (1) at least two contiguous nucleotides nonidentical to the sequences in Table 6, (2) at least three contiguous nucleotides nonidentical to the sequences in Table 6, (3) at least four contiguous nucleotides nonidentical to the sequences in Table 6, (4) at least five contiguous nucleotides nonidentical to the sequences in Table 6, (5) at least six contiguous nucleotides nonidentical to the sequences in Table 6, or (6) at least seven contiguous nucleotides nonidentical to the sequences in Table 6.

In another embodiment the fragment has a size selected from the group consisting of at least: 8 nucleotides, 10 nucleotides, 12 nucleotides, 14 nucleotides, 16 nucleotides, 18 nucleotides, 20, nucleotides, 22 nucleotides, 24 nucleotides, 26 nucleotides, 28 nucleotides, 30 nucleotides, 50 nucleotides, 75 nucleotides, 100 nucleotides, 200 nucleotides, 1000 nucleotides and every integer length therebetween.

In yet another embodiment the molecule encodes a polypeptide which, or a fragment of which, binds a human HLA receptor or a human antibody.

Another aspect of the invention is an expression vector comprising an isolated nucleic acid molecule of the invention described above operably linked to a promoter.

According to one aspect the invention is an expression vector comprising a nucleic acid operably linked to a promoter, wherein the nucleic acid is a NA Group 1, Group 2 or Group 3 molecule. In another aspect the invention is an expression vector comprising a NA Group 1 or Group 2 molecule and a nucleic acid encoding an MHC, preferably HLA, molecule.

In yet another aspect the invention is a host cell transformed or transfected with an expression vector of the invention described above.

In another aspect the invention is a host cell transformed or transfected with an expression vector comprising an isolated nucleic acid molecule of the invention described above operably linked to a promoter, or an expression vector comprising a nucleic acid

operably linked to a promoter, wherein the nucleic acid is a NA Group 1 or 2 molecule and further comprising a nucleic acid encoding HLA.

In another aspect, methods for making the nucleic acids described herein and polypeptides encoded thereby are provided. In some embodiments, the methods include  
5 culturing the host cells and isolating the nucleic acid or polypeptide from the host cells or culture medium. In other embodiments, the methods include providing a non-cell system for transcription and/or translation of a nucleic acid, such a cell-free transcription/translation lysate of rabbit reticulocytes or wheat germ extract. The methods also include introducing the nucleic acid or expression vector into the non-cell system, incubating the system under  
10 conditions sufficient for transcription or translation of the nucleic acid and isolating the transcribed nucleic acid or translated polypeptide from the non-cell system.

According to another aspect of the invention isolated polypeptides encoded by the isolated nucleic acid molecules of the invention, described above, are provided. These include PP Group 1-5 polypeptides. The invention also includes a fragment of the polypeptide  
15 which is immunogenic. In one embodiment the fragment, or a portion of the fragment, binds HLA or a human antibody.

The invention includes in another aspect an isolated fragment of a human cancer associated antigen precursor which, or portion of which, binds HLA or a human antibody, wherein the precursor is encoded by a nucleic acid molecule that is a NA Group 1 molecule.  
20 In one embodiment the fragment is part of a complex with HLA. In another embodiment the fragment is between 8 and 12 amino acids in length. In another embodiment the invention includes an isolated polypeptide comprising a fragment of the polypeptide of sufficient length to represent a sequence unique within the human genome and identifying a polypeptide that is a human cancer associated antigen precursor.

According to another aspect of the invention a kit for detecting the presence of the  
25 expression of a cancer associated antigen precursor is provided. The kit includes a pair of isolated nucleic acid molecules each of which consists essentially of a molecule selected from the group consisting of (a) a 12-32 nucleotide contiguous segment of the nucleotide sequence of any of the NA Group 1 molecules and (b) complements of ("a"), wherein the contiguous  
30 segments are nonoverlapping. In one embodiment the pair of isolated nucleic acid molecules is constructed and arranged to selectively amplify an isolated nucleic acid molecule that is a NA Group 3 molecule. Preferably, the pair amplifies a human NA Group 3 molecule.

According to another aspect of the invention a method for treating a subject with a disorder characterized by expression of a human cancer associated antigen precursor is provided. The method includes the step of administering to the subject an amount of an agent, which enriches selectively in the subject the presence of complexes of an HLA molecule and a human cancer associated antigen, effective to ameliorate the disorder, wherein the human cancer associated antigen is a fragment of a human cancer associated antigen precursor encoded by a nucleic acid molecule selected from the group consisting of (a) a nucleic acid molecule comprising NA group 1 nucleic acid molecules, (b) a nucleic acid molecule comprising NA group 3 nucleic acid molecules and (c) a nucleic acid molecule comprising NA group 5 nucleic acid molecules.

In one embodiment the disorder is characterized by expression of a plurality of human cancer associated antigen precursors and the agent is a plurality of agents, each of which enriches selectively in the subject the presence of complexes of an HLA molecule and a different human cancer associated antigen. Preferably the plurality is at least 2, at least 3, at least 4, or at least 5 such agents.

In another embodiment the agent is an isolated polypeptide selected from the group consisting of PP Group 1, PP Group 2, PP Group 3, PP Group 4, and PP group 5 polypeptides.

In yet another embodiment the disorder is cancer.

According to another aspect the invention is a method for treating a subject having a condition characterized by expression of a cancer associated antigen precursor in cells of the subject. The method includes the steps of (i) removing an immunoreactive cell containing sample from the subject, (ii) contacting the immunoreactive cell containing sample to the host cell under conditions favoring production of cytolytic T cells against a human cancer associated antigen which is a fragment of the precursor, (iii) introducing the cytolytic T cells to the subject in an amount effective to lyse cells which express the human cancer associated antigen, wherein the host cell is transformed or transfected with an expression vector comprising an isolated nucleic acid molecule operably linked to a promoter, the isolated nucleic acid molecule being selected from the group of nucleic acid molecules consisting of NA Group 1, NA Group 2, NA Group 3, NA Group 4, NA Group 5.

In one embodiment the host cell recombinantly expresses an HLA molecule which binds the human cancer associated antigen. In another embodiment the host cell endogenously expresses an HLA molecule which binds the human cancer associated antigen.

The invention includes in another aspect a method for treating a subject having a condition characterized by expression of a cancer associated antigen precursor in cells of the subject. The method includes the steps of (i) identifying a nucleic acid molecule expressed by the cells associated with said condition, wherein said nucleic acid molecule is a NA Group 1 molecule (ii) transfecting a host cell with a nucleic acid selected from the group consisting of (a) the nucleic acid molecule identified, (b) a fragment of the nucleic acid identified which includes a segment coding for a cancer associated antigen, (c) deletions, substitutions or additions to (a) or (b), and (d) degenerates of (a), (b), or (c); (iii) culturing said transfected host cells to express the transfected nucleic acid molecule, and; (iv) introducing an amount of said host cells or an extract thereof to the subject effective to increase an immune response against the cells of the subject associated with the condition. Preferably, the antigen is a human antigen and the subject is a human.

In one embodiment the method also includes the step of (a) identifying an MHC molecule which presents a portion of an expression product of the nucleic acid molecule, wherein the host cell expresses the same MHC molecule as identified in (a) and wherein the host cell presents an MHC binding portion of the expression product of the nucleic acid molecule.

In another embodiment the method also includes the step of treating the host cells to render them non-proliferative.

In yet another embodiment the immune response comprises a B-cell response or a T cell response. Preferably the response is a T-cell response which comprises generation of cytolytic T-cells specific for the host cells presenting the portion of the expression product of the nucleic acid molecule or cells of the subject expressing the human cancer associated antigen.

In another embodiment the nucleic acid molecule is a NA Group 3 molecule.

Another aspect of the invention is a method for treating or diagnosing or monitoring a subject having a condition characterized by expression of an abnormal amount of a protein encoded by a nucleic acid molecule that is a NA Group 1 molecule. The method includes the step of administering to the subject an antibody which specifically binds to the protein or a peptide derived therefrom, the antibody being coupled to a therapeutically useful agent, in an amount effective to treat the condition.

In one embodiment the antibody is a monoclonal antibody. Preferably the monoclonal



antibody is a chimeric antibody or a humanized antibody.

In another aspect the invention is a method for treating a condition characterized by expression in a subject of abnormal amounts of a protein encoded by a nucleic acid molecule that is a NA Group 1 nucleic acid molecule. The method involves the step of administering to  
5 a subject at least one of the pharmaceutical compositions of the invention described above in an amount effective to prevent, delay the onset of, or inhibit the condition in the subject. In one embodiment the condition is cancer. In another embodiment the method includes the step of first identifying that the subject expresses in a tissue abnormal amounts of the protein.

The invention in another aspect is a method for treating a subject having a condition  
10 characterized by expression of abnormal amounts of a protein encoded by a nucleic acid molecule that is a NA Group 1 nucleic acid molecule. The method includes the steps of (i) identifying cells from the subject which express abnormal amounts of the protein; (ii) isolating a sample of the cells; (iii) cultivating the cells, and (iv) introducing the cells to the subject in an amount effective to provoke an immune response against the cells.

15 In one embodiment the method includes the step of rendering the cells non-proliferative, prior to introducing them to the subject.

In another aspect the invention is a method for treating a pathological cell condition characterized by abnormal expression of a protein encoded by a nucleic acid molecule that is a NA Group 1 nucleic acid molecule. The method includes the step of administering to a  
20 subject in need thereof an effective amount of an agent which inhibits the expression or activity of the protein.

In one embodiment the agent is an inhibiting antibody which selectively binds to the protein and wherein the antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody or a fragment thereof. In another embodiment the agent is an antisense nucleic acid  
25 molecule which selectively binds to the nucleic acid molecule which encodes the protein. In yet another important embodiment the nucleic acid molecule is a NA Group 3 nucleic acid molecule.

The invention includes in another aspect a composition of matter useful in stimulating an immune response to a plurality of proteins encoded by nucleic acid molecules that are NA  
30 Group 1 molecules. The composition is a plurality of peptides derived from the amino acid sequences of the proteins, wherein the peptides bind to one or more MHC molecules presented on the surface of the cells which express an abnormal amount of the protein.

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In one embodiment at least a portion of the plurality of peptides bind to MHC molecules and elicit a cytolytic or antibody response thereto. In another embodiment the composition of matter includes an adjuvant and/or a costimulatory molecule. In another embodiment the adjuvant is a saponin, GM-CSF, or an interleukin. In still another embodiment, the compositions also includes at least one peptide useful in stimulating an immune response to at least one protein which is not encoded by nucleic acid molecules that are NA Group 1 molecules, wherein the at least one peptide binds to one or more MHC molecules.

According to another aspect the invention is an isolated antibody which selectively binds to a complex of: (i) a peptide derived from a protein encoded by a nucleic acid molecule that is a NA Group 1 molecule and (ii) and an MHC molecule to which binds the peptide to form the complex, wherein the isolated antibody does not bind to (i) or (ii) alone.

In one embodiment the antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody or a fragment thereof.

The invention also involves the use of the genes, gene products, fragments thereof, agents which bind thereto, and so on in the preparation of medicaments. A particular medicament is for treating cancer and a more particular medicament is for treating seminomas, melanomas, teratomas, gliomas, lung cancer, ovarian cancer and/or colorectal cancer.

#### **Brief Description of the Drawing**

Fig. 1 shows a helical wheel diagram of HOM-TES-85/CT-8 leucine zipper.

#### **Detailed Description of the Invention**

Several new genes which are cancer associated antigens (CTAs), including a novel CTA, HOM-TES-85 (CTA-8), have been cloned and molecularly characterized. These cancer associated antigens were identified by SEREX using a testis-enriched cDNA library and high-titered IgG in the serum of a seminoma patient. Sequence analysis and database search revealed that HOM-TES-85 is a novel leucine zipper protein. It may therefore be involved in DNA-binding and gene transcription in tumor cells. The HOM-TES-85 oncogenic function, and the function of the other cancer associated antigens can be inhibited by specific immunological and genetic therapeutic interventions.

In the above summary and in the ensuing description, lists of sequences are provided. The lists are meant to embrace each single sequence separately, two or more sequences together where they form a part of the same gene, any combination of two or more sequences which relate to different genes, including and up to the total number on the list, as if each and every combination were separately and specifically enumerated. Likewise, when mentioning  
5 fragment size, it is intended that a range embrace the smallest fragment mentioned to the full-length of the sequence (less one nucleotide or amino acid so that it is a fragment), each and every fragment length intended as if specifically enumerated. Thus, if a fragment could be between 10 and 15 in length, it is explicitly meant to mean 10, 11, 12, 13, 14, or 15 in length.

10 The summary and the claims mention antigen precursors and antigens. As used in the summary and in the claims, a precursor is substantially the full-length protein encoded by the coding region of the isolated DNA and the antigen is a peptide which complexes with MHC, preferably HLA, and which participates in the immune response as part of that complex. Such antigens are typically 9 amino acids long (for HLA class II molecules), although this may vary  
15 slightly.

The nucleic acid molecules described herein preferably are isolated. As used herein with respect to nucleic acids, the term "isolated" means: (i) amplified *in vitro* by, for example, polymerase chain reaction (PCR); (ii) recombinantly produced by cloning; (iii) purified, as by cleavage and gel separation; or (iv) synthesized by, for example, chemical synthesis. An  
20 isolated nucleic acid is one which is readily manipulable by recombinant DNA techniques well known in the art. Thus, a nucleotide sequence contained in a vector in which 5' and 3' restriction sites are known or for which polymerase chain reaction (PCR) primer sequences have been disclosed is considered isolated but a nucleic acid sequence existing in its native state in its natural host is not. An isolated nucleic acid may be substantially purified, but need  
25 not be. For example, a nucleic acid that is isolated within a cloning or expression vector is not pure in that it may comprise only a tiny percentage of the material in the cell in which it resides. Such a nucleic acid is isolated, however, as the term is used herein because it is readily manipulable by standard techniques known to those of ordinary skill in the art. An isolated nucleic acid as used herein is not a naturally occurring chromosome.

30 The polypeptides described herein also preferably are isolated. As used herein with respect to polypeptides, "isolated" means separated from its native environment and present in sufficient quantity to permit its identification or use. Isolated, when referring to a protein or

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polypeptide, means, for example: (i) selectively produced by expression cloning or (ii) purified as by chromatography or electrophoresis. Isolated proteins or polypeptides may, but need not be, substantially pure. The term "substantially pure" means that the proteins or polypeptides are essentially free of other substances with which they may be found in nature or *in vivo* systems to an extent practical and appropriate for their intended use. Substantially pure polypeptides may be produced by techniques well known in the art. Because an isolated protein may be admixed with a pharmaceutically acceptable carrier in a pharmaceutical preparation, the protein may comprise only a small percentage by weight of the preparation. The protein is nonetheless isolated in that it has been separated from the substances with which it may be associated in living systems, i.e. isolated from other proteins.

As used herein, a subject is a human, non-human primate, cow, horse, pig, sheep, goat, dog, cat, rodent or other verterinary animal. In all embodiments human cancer antigens and human subjects are preferred.

The present invention in one aspect involves the cloning of cDNAs encoding human cancer associated antigen precursors using autologous antisera of subjects having seminoma cancer. The sequences of the clones representing genes identified according to the methods described herein are presented in the attached Sequence Listing. Of the foregoing, it can be seen that some of the clones are considered completely novel as no nucleotide or amino acid homologies to coding regions were found in the databases searched. Other clones are novel but have some homology to sequences deposited in databases (mainly EST sequences). Nevertheless, the entire gene sequence was not previously known. In some cases no function was suspected and in other cases, even if a function was suspected, it was not know that the gene was associated with cancer. In all cases, it was not known or suspected that the gene encoded a cancer antigen which reacted with antibody from autologous sera. Analysis of the clone sequences by comparison to nucleic acid and protein databases determined that still other of the clones surprisingly are closely related to other previously-cloned genes. The sequences of these related genes is also presented in the Sequence Listing. The nature of the foregoing genes as encoding antigens recognized by the immune systems of cancer patients is, of course, unexpected.

The invention thus involves in one aspect cancer associated antigen polypeptides, genes encoding those polypeptides, functional modifications and variants of the foregoing, useful fragments of the foregoing, as well as diagnostics and therapeutics relating thereto.

Homologs and alleles of the cancer associated antigen nucleic acids of the invention can be identified by conventional techniques. Thus, an aspect of the invention is those nucleic acid sequences which code for cancer associated antigen precursors. Preferred nucleic acid molecules exclude nucleic acid molecules that are composed entirely of the nucleotide sequences of the molecules identified by GenBank accession number in Table 6. Because this application contains several nucleotide and amino acid sequences, the following chart is provided to identify the various groups of sequences discussed in the claims and in the summary:

#### Nucleic Acid Sequences

NA Group 1. (a) nucleic acid molecules which code for a cancer associated antigen precursor and which hybridize under stringent conditions to a molecule consisting of a nucleic acid sequence selected from the group consisting of nucleic acid sequences among SEQ ID

NOs:4-11,

(b) deletions, additions and substitutions which code for a respective cancer associated antigen precursor,

(c) nucleic acid molecules that differ from the nucleic acid molecules of (a) or (b) in codon sequence due to the degeneracy of the genetic code, and

(d) complements of (a), (b) or (c).

NA Group 2. Fragments of NA Group 1, which code for a polypeptide which, or a portion of which, binds an MHC molecule to form a complex recognized by a an autologous antibody or lymphocyte.

NA Group 3. The subset of NA Group 1 where the nucleotide sequence is selected from the group consisting of:

(a) previously unknown human nucleic acids coding for a human cancer associated antigen precursor (i.e. nucleic acid sequences among SEQ ID NOs: 5-10),

(b) deletions, additions and substitutions which code for a respective human cancer associated antigen precursor,

(c) nucleic acid molecules that differ from the nucleic acid molecules of (a) or

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- (b) in codon sequence due to the degeneracy of the genetic code, and  
(d) complements of (a), (b) or (c).

NA Group 4. Fragments of NA Group 3, which code for a polypeptide which, or a portion of  
5 which, binds to an MHC molecule to form a complex recognized by an autologous antibody  
or lymphocyte.

NA Group 5. A subset of NA Group 1, comprising human cancer associated antigens that  
react with allogeneic cancer antisera.

10

#### Polypeptide Sequences

PP Group 1. Polypeptides encoded by NA Group 1.

PP Group 2. Polypeptides encoded by NA Group 2

15 PP Group 3. Polypeptides encoded by NA Group 3.

PP Group 4. Polypeptides encoded by NA Group 4.

PP Group 5. Polypeptides encoded by NA Group 5.

The term "stringent conditions" as used herein refers to parameters with which the art  
20 is familiar. Nucleic acid hybridization parameters may be found in references which compile  
such methods, e.g. *Molecular Cloning: A Laboratory Manual*, J. Sambrook, et al., eds.,  
Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989,  
or *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., eds., John Wiley & Sons,  
Inc., New York. More specifically, stringent conditions, as used herein, refers, for example,  
25 to hybridization at 65°C in hybridization buffer (3.5 x SSC, 0.02% Ficoll, 0.02% polyvinyl  
pyrrolidone, 0.02% Bovine Serum Albumin, 2.5mM NaH<sub>2</sub>PO<sub>4</sub>(pH7), 0.5% SDS, 2mM  
EDTA). SSC is 0.15M sodium chloride/0.15M sodium citrate, pH7; SDS is sodium dodecyl  
sulphate; and EDTA is ethylenediaminetetracetic acid. After hybridization, the membrane  
upon which the DNA is transferred is washed, for example, in 2 x SSC at room temperature  
30 and then at 0.1 - 0.5 x SSC/0.1 x SDS at temperatures up to 68°C.

There are other conditions, reagents, and so forth which can be used, which result in a  
similar degree of stringency. The skilled artisan will be familiar with such conditions, and

thus they are not given here. It will be understood, however, that the skilled artisan will be able to manipulate the conditions in a manner to permit the clear identification of homologs and alleles of cancer associated antigen nucleic acids of the invention (e.g., by using lower stringency conditions). The skilled artisan also is familiar with the methodology for screening  
5 cells and libraries for expression of such molecules which then are routinely isolated, followed by isolation of the pertinent nucleic acid molecule and sequencing.

In general homologs and alleles typically will share at least 40% nucleotide identity and/or at least 50% amino acid identity to the sequences of cancer associated antigen nucleic acid and polypeptides, respectively, in some instances will share at least 50% nucleotide  
10 identity and/or at least 65% amino acid identity and in still other instances will share at least 60% nucleotide identity and/or at least 75% amino acid identity. Preferably the homologs and alleles will share at least 80% nucleotide identity and/or at least 90% amino acid identity, and more preferably will share at least 90% nucleotide identity and/or at least 95% amino acid identity. Most preferably the homologs and alleles will share at least 95% nucleotide identity  
15 and/or at least 99% amino acid identity. The homology can be calculated using various, publicly available software tools developed by NCBI (Bethesda, Maryland) that can be obtained through the internet (<ftp://ncbi.nlm.nih.gov/pub/>). Exemplary tools include the BLAST system available from the National Center for Biotechnology Information at <http://www.ncbi.nlm.nih.gov>, used with default settings. Pairwise and ClustalW alignments  
20 (BLOSUM30 matrix setting) as well as Kyte-Doolittle hydropathic analysis can be obtained, for example, using the MacVector sequence analysis software (Oxford Molecular Group). Watson-Crick complements of the foregoing nucleic acids also are embraced by the invention.

In screening for cancer associated antigen genes, a Southern blot may be performed using the foregoing conditions, together with a radioactive probe. After washing the  
25 membrane to which the DNA is finally transferred, the membrane can be placed against X-ray film to detect the radioactive signal. In screening for the expression of cancer associated antigen nucleic acids, Northern blot hybridizations using the foregoing conditions (see also the Examples) can be performed on samples taken from cancer patients or subjects suspected of having a condition characterized by expression of cancer associated antigen genes.

30 Amplification protocols such as polymerase chain reaction using primers which hybridize to the sequences presented also can be used for detection of the cancer associated antigen genes or expression thereof.

The cancer associated genes correspond to SEQ ID NOs:4-10. The preferred cancer associated antigens for the methods of diagnosis disclosed herein are those which react with allogeneic cancer antisera (i.e. NA Group 5). Encoded polypeptides (e.g., proteins), peptides and antisera thereto are also preferred for diagnosis.

5       The invention also includes degenerate nucleic acids which include alternative codons to those present in the native materials. For example, serine residues are encoded by the codons TCA, AGT, TCC, TCG, TCT and AGC. Each of the six codons is equivalent for the purposes of encoding a serine residue. Thus, it will be apparent to one of ordinary skill in the art that any of the serine-encoding nucleotide triplets may be employed to direct the protein  
10       synthesis apparatus, *in vitro* or *in vivo*, to incorporate a serine residue into an elongating cancer associated antigen polypeptide. Similarly, nucleotide sequence triplets which encode other amino acid residues include, but are not limited to: CCA, CCC, CCG and CCT (proline codons); CGA, CGC, CGG, CGT, AGA and AGG (arginine codons); ACA, ACC, ACG and ACT (threonine codons); AAC and AAT (asparagine codons); and ATA, ATC and ATT  
15       (isoleucine codons). Other amino acid residues may be encoded similarly by multiple nucleotide sequences. Thus, the invention embraces degenerate nucleic acids that differ from the biologically isolated nucleic acids in codon sequence due to the degeneracy of the genetic code.

      The invention also provides modified nucleic acid molecules which include additions,  
20       substitutions and deletions of one or more nucleotides. In preferred embodiments, these modified nucleic acid molecules and/or the polypeptides they encode retain at least one activity or function of the unmodified nucleic acid molecule and/or the polypeptides, such as antigenicity, enzymatic activity, receptor binding, formation of complexes by binding of peptides by MHC class I and class II molecules, etc. In certain embodiments, the modified  
25       nucleic acid molecules encode modified polypeptides, preferably polypeptides having conservative amino acid substitutions as are described elsewhere herein. The modified nucleic acid molecules are structurally related to the unmodified nucleic acid molecules and in preferred embodiments are sufficiently structurally related to the unmodified nucleic acid molecules so that the modified and unmodified nucleic acid molecules hybridize under  
30       stringent conditions known to one of skill in the art.

      For example, modified nucleic acid molecules which encode polypeptides having single amino acid changes can be prepared. Each of these nucleic acid molecules can have



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one, two or three nucleotide substitutions exclusive of nucleotide changes corresponding to the degeneracy of the genetic code as described herein. Likewise, modified nucleic acid molecules which encode polypeptides having two amino acid changes can be prepared which have, e.g., 2-6 nucleotide changes. Numerous modified nucleic acid molecules like these will be readily envisioned by one of skill in the art, including for example, substitutions of nucleotides in codons encoding amino acids 2 and 3, 2 and 4, 2 and 5, 2 and 6, and so on. In the foregoing example, each combination of two amino acids is included in the set of modified nucleic acid molecules, as well as all nucleotide substitutions which code for the amino acid substitutions. Additional nucleic acid molecules that encode polypeptides having additional substitutions (i.e., 3 or more), additions or deletions (e.g., by introduction of a stop codon or a splice site(s)) also can be prepared and are embraced by the invention as readily envisioned by one of ordinary skill in the art. Any of the foregoing nucleic acids or polypeptides can be tested by routine experimentation for retention of structural relation or activity to the nucleic acids and/or polypeptides disclosed herein.

The invention also provides isolated unique fragments of cancer associated antigen nucleic acid sequences or complements thereof. A unique fragment is one that is a 'signature' for the larger nucleic acid. It, for example, is long enough to assure that its precise sequence is not found in molecules within the human genome outside of the cancer associated antigen nucleic acids defined above (and human alleles). Those of ordinary skill in the art may apply no more than routine procedures to determine if a fragment is unique within the human genome. Unique fragments, however, exclude fragments completely composed of the nucleotide sequences of any of GenBank accession numbers listed in Table 6 or other previously published sequences as of the filing date of the priority documents for sequences listed in a respective priority document or the filing date of this application for sequences listed for the first time in this application which overlap the sequences of the invention.

A fragment which is completely composed of the sequence described in the foregoing GenBank deposits is one which does not include any of the nucleotides unique to the sequences of the invention. Thus, a unique fragment must contain a nucleotide sequence other than the exact sequence of those in GenBank or fragments thereof. The difference may be an addition, deletion or substitution with respect to the GenBank sequence or it may be a sequence wholly separate from the GenBank sequence.

Unique fragments can be used as probes in Southern and Northern blot assays to

identify such nucleic acids, or can be used in amplification assays such as those employing PCR. As known to those skilled in the art, large probes such as 200, 250, 300 or more nucleotides are preferred for certain uses such as Southern and Northern blots, while smaller fragments will be preferred for uses such as PCR. Unique fragments also can be used to produce fusion proteins for generating antibodies or determining binding of the polypeptide fragments, or for generating immunoassay components. Likewise, unique fragments can be employed to produce nonfused fragments of the cancer associated antigen polypeptides, useful, for example, in the preparation of antibodies, and in immunoassays. Unique fragments further can be used as antisense molecules to inhibit the expression of cancer associated antigen nucleic acids and polypeptides, particularly for therapeutic purposes as described in greater detail below.

As will be recognized by those skilled in the art, the size of the unique fragment will depend upon its conservancy in the genetic code. Thus, some regions of cancer associated antigen sequences and complements thereof will require longer segments to be unique (e.g., 50, 75, 100, 150, 200, 250, 300, 400, 500, 750, 1000 nucleotides) while others will require only short segments, typically between 12 and 32 nucleotides (e.g. 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32), up to the entire length of the disclosed sequence. As mentioned above, this disclosure intends to embrace each and every fragment of each sequence, beginning at the first nucleotide, the second nucleotide and so on, up to 8 nucleotides short of the end, and ending anywhere from nucleotide number 8, 9, 10 and so on for each sequence, up to the very last nucleotide (provided the sequence is unique as described above).

Virtually any segment of the polypeptide coding region of novel cancer associated antigen nucleic acids, or complements thereof, that is 25 or more nucleotides in length will be unique. Those skilled in the art are well versed in methods for selecting such sequences, typically on the basis of the ability of the unique fragment to selectively distinguish the sequence of interest from other sequences in the human genome of the fragment to those on known databases typically is all that is necessary, although *in vitro* confirmatory hybridization and sequencing analysis may be performed.

Especially preferred nucleic acid molecules include nucleic acids encoding a series of epitopes, known as "polytopes". The epitopes can be arranged in sequential or overlapping fashion (see, e.g., Thomson et al., *Proc. Natl. Acad. Sci. USA* 92:5845-5849, 1995; Gilbert et

al., *Nature Biotechnol.* 15:1280-1284, 1997), with or without the natural flanking sequences, and can be separated by unrelated linker sequences if desired. The polytope is processed to generate individual epitopes which are recognized by the immune system for generation of immune responses.

5 Thus, for example, peptides derived from a polypeptide having an amino acid sequence encoded by one of the nucleic acid molecules disclosed herein, and which are presented by MHC molecules and recognized by CTL or T helper lymphocytes, can be combined with peptides from one or more other cancer associated antigens (e.g. by preparation of hybrid nucleic acids or polypeptides) to form "polytopes". The two or more  
10 peptides (or nucleic acids encoding the peptides) can be selected from those described herein, or they can include one or more peptides of previously known cancer associated antigens. Exemplary cancer associated peptide antigens (which are presented by MHC class I or II) that can be administered to induce or enhance an immune response are derived from tumor associated genes and encoded proteins including MAGE-A1, MAGE-A2, MAGE-A3,  
15 MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9, BAGE-1, RAGE-1, LB33/MUM-1, PRAME, NAG, MAGE-B2, MAGE-B3, MAGE-B4, tyrosinase, brain glycogen phosphorylase, Melan-A, MAGE-C1, MAGE-C2, NY-ESO-1, LAGE-1, SSX-1, SSX-2 (HOM-MEL-40), SSX-4, SSX-5, SCP-1  
20 and CT-7. See, for example, PCT application publication no. WO96/10577. Other examples will be known to one of ordinary skill in the art (for example, see Coulie, *Stem Cells* 13:393-403, 1995), and can be used in the invention in a like manner as those disclosed herein. One of ordinary skill in the art can prepare polypeptides comprising one or more peptides of the invention and one or more of the foregoing known cancer associated peptides, or nucleic acids  
25 encoding such polypeptides, according to standard procedures of molecular biology.

Thus polytopes are groups of two or more potentially immunogenic or immune response stimulating peptides which can be joined together in various arrangements (e.g. concatenated, overlapping). The polytope (or nucleic acid encoding the polytope) can be administered in a standard immunization protocol, e.g. to animals, to test the effectiveness of  
30 the polytope in stimulating, enhancing and/or provoking an immune response.

The peptides can be joined together directly or via the use of flanking sequences to form polytopes, and the use of polytopes as vaccines is well known in the art (see, e.g.,

Thomson et al., *Proc. Acad. Natl. Acad. Sci USA* 92(13):5845-5849, 1995; Gilbert et al., *Nature Biotechnol.* 15(12):1280-1284, 1997; Thomson et al., *J. Immunol.* 157(2):822-826, 1996; Tam et al., *J. Exp. Med.* 171(1):299-306, 1990). For example, Tam showed that polytopes consisting of both MHC class I and class II binding epitopes successfully generated antibody and protective immunity in a mouse model. Tam also demonstrated that polytopes comprising "strings" of epitopes are processed to yield individual epitopes which are presented by MHC molecules and recognized by CTLs. Thus polytopes containing various numbers and combinations of epitopes can be prepared and tested for recognition by CTLs and for efficacy in increasing an immune response.

It is known that tumors express a set of tumor antigens, of which only certain subsets may be expressed in the tumor of any given patient. Polytopes can be prepared which correspond to the different combination of epitopes representing the subset of tumor rejection antigens expressed in a particular patient. Polytopes also can be prepared to reflect a broader spectrum of tumor rejection antigens known to be expressed by a tumor type. Polytopes can be introduced to a patient in need of such treatment as polypeptide structures, or via the use of nucleic acid delivery systems known in the art (see, e.g., Allsopp et al., *Eur. J. Immunol.* 26(8):1951-1959, 1996). Adenovirus, pox virus, Ty-virus like particles, adeno-associated virus, plasmids, bacteria, etc. can be used in such delivery. One can test the polytope delivery systems in mouse models to determine efficacy of the delivery system. The systems also can be tested in human clinical trials.

In instances in which a human HLA molecule presents tumor rejection antigens derived from cancer associated nucleic acids, the expression vector may also include a nucleic acid sequence coding for the HLA molecule that presents any particular tumor rejection antigen derived from these nucleic acids and polypeptides. Alternatively, the nucleic acid sequence coding for such a HLA molecule can be contained within a separate expression vector. In a situation where the vector contains both coding sequences, the single vector can be used to transfect a cell which does not normally express either one. Where the coding sequences for a cancer associated antigen precursor and the HLA molecule which presents it are contained on separate expression vectors, the expression vectors can be cotransfected. The cancer associated antigen precursor coding sequence may be used alone, when, e.g. the host cell already expresses a HLA molecule which presents a cancer associated antigen derived from precursor molecules. Of course, there is no limit on the particular host cell

which can be used. As the vectors which contain the two coding sequences may be used in any antigen-presenting cells if desired, and the gene for cancer associated antigen precursor can be used in host cells which do not express a HLA molecule which presents a cancer associated antigen. Further, cell-free transcription systems may be used in lieu of cells.

5 As used herein, a "vector" may be any of a number of nucleic acids into which a desired sequence may be inserted by restriction and ligation for transport between different genetic environments or for expression in a host cell. Vectors are typically composed of DNA although RNA vectors are also available. Vectors include, but are not limited to, plasmids, phagemids and virus genomes. A cloning vector is one which is able to replicate  
10 autonomously or integrated in the genome in a host cell, and which is further characterized by one or more endonuclease restriction sites at which the vector may be cut in a determinable fashion and into which a desired DNA sequence may be ligated such that the new recombinant vector retains its ability to replicate in the host cell. In the case of plasmids, replication of the desired sequence may occur many times as the plasmid increases in copy  
15 number within the host bacterium or just a single time per host before the host reproduces by mitosis. In the case of phage, replication may occur actively during a lytic phase or passively during a lysogenic phase. An expression vector is one into which a desired DNA sequence may be inserted by restriction and ligation such that it is operably joined to regulatory sequences and may be expressed as an RNA transcript. Vectors may further contain one or  
20 more marker sequences suitable for use in the identification of cells which have or have not been transformed or transfected with the vector. Markers include, for example, genes encoding proteins which increase or decrease either resistance or sensitivity to antibiotics or other compounds, genes which encode enzymes whose activities are detectable by standard assays known in the art (e.g.,  $\beta$ -galactosidase, luciferase or alkaline phosphatase), and genes  
25 which visibly affect the phenotype of transformed or transfected cells, hosts, colonies or plaques (e.g., green fluorescent protein). Preferred vectors are those capable of autonomous replication and expression of the structural gene products present in the DNA segments to which they are operably joined.

As used herein, a coding sequence and regulatory sequences are said to be "operably"  
30 joined when they are covalently linked in such a way as to place the expression or transcription of the coding sequence under the influence or control of the regulatory sequences. If it is desired that the coding sequences be translated into a functional protein,

two DNA sequences are said to be operably joined if induction of a promoter in the 5' regulatory sequences results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the coding sequences, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a promoter region would be operably joined to a coding sequence if the promoter region were capable of effecting transcription of that DNA sequence such that the resulting transcript might be translated into the desired protein or polypeptide.

The precise nature of the regulatory sequences needed for gene expression may vary between species or cell types, but shall in general include, as necessary, 5' non-transcribed and 5' non-translated sequences involved with the initiation of transcription and translation respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribed regulatory sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined gene.

Regulatory sequences may also include enhancer sequences or upstream activator sequences as desired. The vectors of the invention may optionally include 5' leader or signal sequences. The choice and design of an appropriate vector is within the ability and discretion of one of ordinary skill in the art.

Expression vectors containing all the necessary elements for expression are commercially available and known to those skilled in the art. See, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, 1989. Cells are genetically engineered by the introduction into the cells of heterologous DNA (RNA) encoding a cancer associated antigen polypeptide or fragment or variant thereof. That heterologous DNA (RNA) is placed under operable control of transcriptional elements to permit the expression of the heterologous DNA in the host cell.

Preferred systems for mRNA expression in mammalian cells are those such as pcDNA3.1 and pRc/CMV (available from Invitrogen, Carlsbad, CA) that contain a selectable marker such as a gene that confers G418 resistance (which facilitates the selection of stably transfected cell lines) and the human cytomegalovirus (CMV) enhancer-promoter sequences. Additionally, suitable for expression in primate or canine cell lines is the pCEP4 vector (Invitrogen), which contains an Epstein Barr Virus (EBV) origin of replication, facilitating the maintenance of plasmid as a multicopy extrachromosomal element. Another expression

vector is the pEF-BOS plasmid containing the promoter of polypeptide Elongation Factor 1 $\alpha$ , which stimulates efficiently transcription *in vitro*. The plasmid is described by Mishizuma and Nagata (*Nuc. Acids Res.* 18:5322, 1990), and its use in transfection experiments is disclosed by, for example, Demoulin (*Mol. Cell. Biol.* 16:4710-4716, 1996). Still another preferred expression vector is an adenovirus, described by Stratford-Perricaudet, which is defective for E1 and E3 proteins (*J. Clin. Invest.* 90:626-630, 1992). The use of the adenovirus as an Adeno.P1A recombinant for the expression of an antigen is disclosed by Warnier et al., in intradermal injection in mice for immunization against P1A (*Int. J. Cancer*, 67:303-310, 1996). Additional vectors for delivery of nucleic acid are provided below.

The invention also embraces so-called expression kits, which allow the artisan to prepare a desired expression vector or vectors. Such expression kits include at least separate portions of a vector and one or more of the previously discussed cancer associated antigen nucleic acid molecules. Other components may be added, as desired, as long as the previously mentioned nucleic acid molecules, which are required, are included. The invention also includes kits for amplification of a cancer associated antigen nucleic acid, including at least one pair of amplification primers which hybridize to a cancer associated antigen nucleic acid. The primers preferably are 12-32 nucleotides in length and are non-overlapping to prevent formation of "primer-dimers". One of the primers will hybridize to one strand of the cancer associated antigen nucleic acid and the second primer will hybridize to the complementary strand of the cancer associated antigen nucleic acid, in an arrangement which permits amplification of the cancer associated antigen nucleic acid. Selection of appropriate primer pairs is standard in the art. For example, the selection can be made with assistance of a computer program designed for such a purpose, optionally followed by testing the primers for amplification specificity and efficiency.

The invention also permits the construction of cancer associated antigen gene "knock-outs" and stable or transient transgenic expression in cells and in animals, providing materials for studying certain aspects of cancer and immune system responses to cancer.

As mentioned above, the invention embraces antisense oligonucleotides that selectively bind to a nucleic acid molecule encoding a cancer associated antigen polypeptide, to reduce the expression of cancer associated antigens. This is desirable in virtually any medical condition wherein a reduction of expression of cancer associated antigens is desirable, e.g., in the treatment of cancer. This is also useful for *in vitro* or *in vivo* testing of

the effects of a reduction of expression of one or more cancer associated antigens.

As used herein, the term "antisense oligonucleotide" or "antisense" describes an oligonucleotide that is an oligoribonucleotide, oligodeoxyribonucleotide, modified oligoribonucleotide, or modified oligodeoxyribonucleotide which hybridizes under physiological conditions to DNA comprising a particular gene or to an mRNA transcript of that gene and, thereby, inhibits the transcription of that gene and/or the translation of that mRNA. The antisense molecules are designed so as to interfere with transcription or translation of a target gene upon hybridization with the target gene or transcript. Those skilled in the art will recognize that the exact length of the antisense oligonucleotide and its degree of complementarity with its target will depend upon the specific target selected, including the sequence of the target and the particular bases which comprise that sequence. It is preferred that the antisense oligonucleotide be constructed and arranged so as to bind selectively with the target under physiological conditions, i.e., to hybridize substantially more to the target sequence than to any other sequence in the target cell under physiological conditions. Based upon the sequences of nucleic acids encoding cancer associated antigen, or upon allelic or homologous genomic and/or cDNA sequences, one of skill in the art can easily choose and synthesize any of a number of appropriate antisense molecules for use in accordance with the present invention. In order to be sufficiently selective and potent for inhibition, such antisense oligonucleotides should comprise at least 10 and, more preferably, at least 15 consecutive bases which are complementary to the target, although in certain cases modified oligonucleotides as short as 7 bases in length have been used successfully as antisense oligonucleotides (Wagner et al., *Nature Biotechnol.* 14:840-844, 1996). Most preferably, the antisense oligonucleotides comprise a complementary sequence of 20-30 bases.

Although oligonucleotides may be chosen which are antisense to any region of the gene or mRNA transcripts, in preferred embodiments the antisense oligonucleotides correspond to N-terminal or 5' upstream sites such as translation initiation, transcription initiation or promoter sites. In addition, 3'-untranslated regions may be targeted. Targeting to mRNA splicing sites has also been used in the art but may be less preferred if alternative mRNA splicing occurs. In addition, the antisense is targeted, preferably, to sites in which mRNA secondary structure is not expected (see, e.g., Sainio et al., *Cell Mol. Neurobiol.* 14(5):439-457, 1994) and at which proteins are not expected to bind. Finally, although the listed sequences are cDNA sequences, one of ordinary skill in the art may easily derive the



genomic DNA corresponding to the cDNA of a cancer associated antigen. Thus, the present invention also provides for antisense oligonucleotides which are complementary to the genomic DNA corresponding to nucleic acids encoding cancer associated antigens. Similarly, antisense to allelic or homologous cDNAs and genomic DNAs are enabled without undue experimentation.

In one set of embodiments, the antisense oligonucleotides of the invention may be composed of "natural" deoxyribonucleotides, ribonucleotides, or any combination thereof. That is, the 5' end of one native nucleotide and the 3' end of another native nucleotide may be covalently linked, as in natural systems, via a phosphodiester internucleoside linkage. These oligonucleotides may be prepared by art recognized methods which may be carried out manually or by an automated synthesizer. They also may be produced recombinantly by vectors.

In preferred embodiments, however, the antisense oligonucleotides of the invention also may include "modified" oligonucleotides. That is, the oligonucleotides may be modified in a number of ways which do not prevent them from hybridizing to their target but which enhance their stability or targeting or which otherwise enhance their therapeutic effectiveness.

The term "modified oligonucleotide" as used herein describes an oligonucleotide in which (1) at least two of its nucleotides are covalently linked via a synthetic internucleoside linkage (i.e., a linkage other than a phosphodiester linkage between the 5' end of one nucleotide and the 3' end of another nucleotide) and/or (2) a chemical group not normally associated with nucleic acids has been covalently attached to the oligonucleotide. Preferred synthetic internucleoside linkages are phosphorothioates, alkylphosphonates, phosphorodithioates, phosphate esters, alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate triesters, acetamidates, carboxymethyl esters and peptides.

The term "modified oligonucleotide" also encompasses oligonucleotides with a covalently modified base and/or sugar. For example, modified oligonucleotides include oligonucleotides having backbone sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position. Thus modified oligonucleotides may include a 2'-O-alkylated ribose group. In addition, modified oligonucleotides may include sugars such as arabinose instead of ribose. The present invention, thus, contemplates pharmaceutical preparations containing modified antisense molecules that are complementary to and

hybridizable with, under physiological conditions, nucleic acids encoding cancer associated antigen polypeptides, together with pharmaceutically acceptable carriers.

Antisense oligonucleotides may be administered as part of a pharmaceutical composition. Such a pharmaceutical composition may include the antisense oligonucleotides in combination with any standard physiologically and/or pharmaceutically acceptable carriers which are known in the art. The compositions should be sterile and contain a therapeutically effective amount of the antisense oligonucleotides in a unit of weight or volume suitable for administration to a patient. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. The term "physiologically acceptable" refers to a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism. The characteristics of the carrier will depend on the route of administration. Physiologically and pharmaceutically acceptable carriers include diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials which are well known in the art, as further described below.

The invention also provides isolated polypeptides (including whole proteins and partial proteins) encoded by the foregoing cancer associated antigen nucleic acids. Such polypeptides are useful, for example, alone or as fusion proteins to generate antibodies, as components of an immunoassay or diagnostic assay or as therapeutics. Cancer associated antigen polypeptides can be isolated from biological samples including tissue or cell homogenates, and can also be expressed recombinantly in a variety of prokaryotic and eukaryotic expression systems by constructing an expression vector appropriate to the expression system, introducing the expression vector into the expression system, and isolating the recombinantly expressed protein. Short polypeptides, including antigenic peptides (such as are presented by MHC molecules on the surface of a cell for immune recognition) also can be synthesized chemically using well-established methods of peptide synthesis.

A unique fragment of a cancer associated antigen polypeptide, in general, has the features and characteristics of unique fragments as discussed above in connection with nucleic acids. As will be recognized by those skilled in the art, the size of the unique fragment will depend upon factors such as whether the fragment constitutes a portion of a conserved protein domain. Thus, some regions of cancer associated antigens will require longer segments to be unique (e.g. 15, 20, 25, 30, 40, 50, 75 or 100 or more amino acids including each integer up to the full length) while others will require only short segments, typically between 5 and 12

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amino acids (e.g. 5, 6, 7, 8, 9, 10, 11 or 12).

Unique fragments of a polypeptide preferably are those fragments which retain a distinct functional capability of the polypeptide. Functional capabilities which can be retained in a unique fragment of a polypeptide include interaction with antibodies, interaction with  
5 other polypeptides or fragments thereof, selective binding of nucleic acids or proteins, and enzymatic activity. One important activity is the ability to act as a signature for identifying the polypeptide. Another is the ability to complex with HLA and to provoke in a human an immune response. Those skilled in the art are well versed in methods for selecting unique amino acid sequences, typically on the basis of the ability of the unique fragment to  
10 selectively distinguish the sequence of interest from non-family members. A comparison of the sequence of the fragment to those on known databases typically is all that is necessary.

The invention embraces variants of the cancer associated antigen polypeptides described above. As used herein, a "variant" of a cancer associated antigen polypeptide is a polypeptide which contains one or more modifications to the primary amino acid sequence of  
15 a cancer associated antigen polypeptide. Modifications which create a cancer associated antigen variant can be made to a cancer associated antigen polypeptide 1) to reduce or eliminate an activity of a cancer associated antigen polypeptide; 2) to enhance a property of a cancer associated antigen polypeptide, such as protein stability in an expression system or the stability of protein-protein binding; 3) to provide a novel activity or property to a cancer  
20 associated antigen polypeptide, such as addition of an antigenic epitope or addition of a detectable moiety; or 4) to provide equivalent or better binding to an HLA molecule. Modifications to a cancer associated antigen polypeptide are typically made to the nucleic acid which encodes the cancer associated antigen polypeptide, and can include deletions, point mutations, truncations, amino acid substitutions and additions of amino acids or non-amino  
25 acid moieties. Alternatively, modifications can be made directly to the polypeptide, such as by cleavage, addition of a linker molecule, addition of a detectable moiety, such as biotin, addition of a fatty acid, and the like. Modifications also embrace fusion proteins comprising all or part of the cancer associated antigen amino acid sequence. One of skill in the art will be familiar with methods for predicting the effect on protein conformation of a change in protein  
30 sequence, and can thus "design" a variant cancer associated antigen polypeptide according to known methods. One example of such a method is described by Dahiyat and Mayo in *Science* 278:82-87, 1997, whereby proteins can be designed *de novo*. The method can be applied to a

known protein to vary a only a portion of the polypeptide sequence. By applying the computational methods of Dahiyat and Mayo, specific variants of a cancer associated antigen polypeptide can be proposed and tested to determine whether the variant retains a desired conformation.

5 In general, variants include cancer associated antigen polypeptides which are modified specifically to alter a feature of the polypeptide unrelated to its desired physiological activity. For example, cysteine residues can be substituted or deleted to prevent unwanted disulfide linkages. Similarly, certain amino acids can be changed to enhance expression of a cancer associated antigen polypeptide by eliminating proteolysis by proteases in an expression system  
10 (e.g., dibasic amino acid residues in yeast expression systems in which KEX2 protease activity is present).

Mutations of a nucleic acid which encode a cancer associated antigen polypeptide preferably preserve the amino acid reading frame of the coding sequence, and preferably do not create regions in the nucleic acid which are likely to hybridize to form secondary  
15 structures, such a hairpins or loops, which can be deleterious to expression of the variant polypeptide.

Mutations can be made by selecting an amino acid substitution, or by random mutagenesis of a selected site in a nucleic acid which encodes the polypeptide. Variant polypeptides are then expressed and tested for one or more activities to determine which  
20 mutation provides a variant polypeptide with the desired properties. Further mutations can be made to variants (or to non-variant cancer associated antigen polypeptides) which are silent as to the amino acid sequence of the polypeptide, but which provide preferred codons for translation in a particular host. The preferred codons for translation of a nucleic acid in, e.g., *E. coli*, are well known to those of ordinary skill in the art. Still other mutations can be made  
25 to the noncoding sequences of a cancer associated antigen gene or cDNA clone to enhance expression of the polypeptide. The activity of variants of cancer associated antigen polypeptides can be tested by cloning the gene encoding the variant cancer associated antigen polypeptide into a bacterial or mammalian expression vector, introducing the vector into an appropriate host cell, expressing the variant cancer associated antigen polypeptide, and testing  
30 for a functional capability of the cancer associated antigen polypeptides as disclosed herein. For example, the variant cancer associated antigen polypeptide can be tested for reaction with autologous or allogeneic sera as disclosed in the Examples. Preparation of other variant

polypeptides may favor testing of other activities, as will be known to one of ordinary skill in the art.

The skilled artisan will also realize that conservative amino acid substitutions may be made in cancer associated antigen polypeptides to provide functional variants of the foregoing polypeptides, i.e., variants that retain the functional capabilities of the cancer associated antigen polypeptides such as stimulation of an immune response, binding to HLA molecules, etc. As used herein, a "conservative amino acid substitution" refers to an amino acid substitution which does not alter the relative charge or size characteristics of the protein in which the amino acid substitution is made. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, e.g. *Molecular Cloning: A Laboratory Manual*, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Exemplary functional variants of the cancer associated antigen polypeptides include conservative amino acid substitutions of in the amino acid sequences of proteins disclosed herein. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

For example, upon determining that a peptide derived from a cancer associated antigen polypeptide is presented by an MHC molecule and recognized by CTLs (e.g., as described in the Examples), one can make conservative amino acid substitutions to the amino acid sequence of the peptide, particularly at residues which are thought not to be direct contact points with the MHC molecule. For example, methods for identifying functional variants of HLA class II binding peptides are provided in a published PCT application of Strominger and Wucherpfennig (PCT/US96/03182). Peptides bearing one or more amino acid substitutions also can be tested for concordance with known HLA/MHC motifs prior to synthesis using, e.g. the computer program described by D'Amato and Drijfhout (D'Amato et al., *Human Immunol.* 43:13-18, 1995; Drijfhout et al., *Human Immunol.* 43:1-12, 1995). The substituted peptides can then be tested for binding to the MHC molecule and recognition by CTLs when bound to MHC. These variants can be tested for improved stability and are useful, *inter alia*, in vaccine compositions.

Conservative amino-acid substitutions in the amino acid sequence of cancer associated

antigen polypeptides to produce functional variants of cancer associated antigen polypeptides typically are made by alteration of a nucleic acid encoding a cancer associated antigen polypeptide. Such substitutions can be made by a variety of methods known to one of ordinary skill in the art. For example, amino acid substitutions may be made by PCR-directed mutation, site-directed mutagenesis according to the method of Kunkel (Kunkel, *Proc. Nat. Acad. Sci. U.S.A.* 82: 488-492, 1985), or by chemical synthesis of a gene encoding a cancer associated antigen polypeptide. Where amino acid substitutions are made to a small unique fragment of a cancer associated antigen polypeptide, such as an antigenic epitope recognized by autologous or allogeneic sera or cytolytic T lymphocytes, the substitutions can be made by directly synthesizing the peptide. The activity of functionally equivalent fragments of cancer associated antigen polypeptides can be tested by cloning the gene encoding the altered cancer associated antigen polypeptide into a bacterial or mammalian expression vector, introducing the vector into an appropriate host cell, expressing the altered cancer associated antigen polypeptide, and testing for a functional capability of the cancer associated antigen polypeptides as disclosed herein. Peptides which are chemically synthesized can be tested directly for function, e.g., for binding to antisera recognizing associated antigens.

The invention as described herein has a number of uses, some of which are described elsewhere herein. First, the invention permits isolation of the cancer associated antigen protein molecules. A variety of methodologies well-known to the skilled practitioner can be utilized to obtain isolated cancer associated antigen molecules. The polypeptide may be purified from cells which naturally produce the polypeptide by chromatographic means or immunological recognition. Alternatively, an expression vector may be introduced into cells to cause production of the polypeptide. In another method, mRNA transcripts may be microinjected or otherwise introduced into cells to cause production of the encoded polypeptide. Translation of mRNA in cell-free extracts such as the reticulocyte lysate system also may be used to produce polypeptide. Those skilled in the art also can readily follow known methods for isolating cancer associated antigen polypeptides. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography and immune-affinity chromatography.

The isolation and identification of cancer associated antigen genes also makes it possible for the artisan to diagnose a disorder characterized by expression of cancer associated antigens. These methods involve determining expression of one or more cancer associated

antigen nucleic acids, and/or encoded cancer associated antigen polypeptides and/or peptides derived therefrom. In the former situation, such determinations can be carried out via any standard nucleic acid determination assay, including the polymerase chain reaction, or assaying with labeled hybridization probes. In the latter situation, such determinations can be carried out by screening patient antisera for recognition of the polypeptide.

The invention also makes it possible isolate proteins which bind to cancer associated antigens as disclosed herein, including antibodies and cellular binding partners of the cancer associated antigens. Additional uses are described further herein.

The invention also provides, in certain embodiments, "dominant negative" polypeptides derived from cancer associated antigen polypeptides. A dominant negative polypeptide is an inactive variant of a protein, which, by interacting with the cellular machinery, displaces an active protein from its interaction with the cellular machinery or competes with the active protein, thereby reducing the effect of the active protein. For example, a dominant negative receptor which binds a ligand but does not transmit a signal in response to binding of the ligand can reduce the biological effect of expression of the ligand. Likewise, a dominant negative catalytically-inactive kinase which interacts normally with target proteins but does not phosphorylate the target proteins can reduce phosphorylation of the target proteins in response to a cellular signal. Similarly, a dominant negative transcription factor which binds to a promoter site in the control region of a gene but does not increase gene transcription can reduce the effect of a normal transcription factor by occupying promoter binding sites without increasing transcription.

The end result of the expression of a dominant negative polypeptide in a cell is a reduction in function of active proteins. One of ordinary skill in the art can assess the potential for a dominant negative variant of a protein, and using standard mutagenesis techniques to create one or more dominant negative variant polypeptides. For example, given the teachings contained herein of cancer associated antigens, especially those which are similar to known proteins which have known activities, one of ordinary skill in the art can modify the sequence of the cancer associated antigens by site-specific mutagenesis, scanning mutagenesis, partial gene deletion or truncation, and the like. See, e.g., U.S. Patent No. 5,580,723 and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, 1989. The skilled artisan then can test the population of mutagenized polypeptides for diminution in a selected and/or for retention of such an

activity. Other similar methods for creating and testing dominant negative variants of a protein will be apparent to one of ordinary skill in the art.

The invention also involves agents such as polypeptides which bind to cancer associated antigen polypeptides. Such binding agents can be used, for example, in screening assays to detect the presence or absence of cancer associated antigen polypeptides and complexes of cancer associated antigen polypeptides and their binding partners and in purification protocols to isolated cancer associated antigen polypeptides and complexes of cancer associated antigen polypeptides and their binding partners. Such agents also can be used to inhibit the native activity of the cancer associated antigen polypeptides, for example, by binding to such polypeptides.

The invention, therefore, embraces peptide binding agents which, for example, can be antibodies or fragments of antibodies having the ability to selectively bind to cancer associated antigen polypeptides. Antibodies include polyclonal and monoclonal antibodies, prepared according to conventional methodology.

Significantly, as is well-known in the art, only a small portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see, in general, Clark, W.R. (1986) The Experimental Foundations of Modern Immunology Wiley & Sons, Inc., New York; Roitt, I. (1991) Essential Immunology, 7th Ed., Blackwell Scientific Publications, Oxford). The pFc' and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an F(ab')<sub>2</sub> fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Proceeding further, Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

Within the antigen-binding portion of an antibody, as is well-known in the art, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the



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paratope (see, in general, Clark, 1986; Roitt, 1991). In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDR3). The CDRs, and in particular the CDR3 regions, and more particularly the heavy chain CDR3, are largely responsible for antibody specificity.

It is now well-established in the art that the non-CDR regions of a mammalian antibody may be replaced with similar regions of conspecific or heterospecific antibodies while retaining the epitopic specificity of the original antibody. This is most clearly manifested in the development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody. See, e.g., U.S. patents 4,816,567, 5,225,539, 5,585,089, 5,693,762 and 5,859,205.

Thus, for example, PCT International Publication Number WO 92/04381 teaches the production and use of humanized murine RSV antibodies in which at least a portion of the murine FR regions have been replaced by FR regions of human origin. Such antibodies, including fragments of intact antibodies with antigen-binding ability, are often referred to as "chimeric" antibodies.

Thus, as will be apparent to one of ordinary skill in the art, the present invention also provides for F(ab')<sub>2</sub>, Fab, Fv and Fd fragments; chimeric antibodies in which the Fc and/or FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric F(ab')<sub>2</sub> fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric Fab fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; and chimeric Fd fragment antibodies in which the FR and/or CDR1 and/or CDR2 regions have been replaced by homologous human or non-human sequences. The present invention also includes so-called single chain antibodies.

Thus, the invention involves polypeptides of numerous size and type that bind specifically to cancer associated antigen polypeptides, and complexes of both cancer associated antigen polypeptides and their binding partners. These polypeptides may be derived also from sources other than antibody technology. For example, such polypeptide binding agents can be provided by degenerate peptide libraries which can be readily prepared in solution, in immobilized form or as phage display libraries. Combinatorial libraries also

can be synthesized of peptides containing one or more amino acids. Libraries further can be synthesized of peptoids and non-peptide synthetic moieties.

Phage display can be particularly effective in identifying binding peptides useful according to the invention. Briefly, one prepares a phage library (using e.g. m13, fd, or  
5 lambda phage), displaying inserts from 4 to about 80 amino acid residues using conventional procedures. The inserts may represent, for example, a completely degenerate or biased array. One then can select phage-bearing inserts which bind to the cancer associated antigen polypeptide. This process can be repeated through several cycles of reselection of phage that bind to the cancer associated antigen polypeptide. Repeated rounds lead to enrichment of  
10 phage bearing particular sequences. DNA sequence analysis can be conducted to identify the sequences of the expressed polypeptides. The minimal linear portion of the sequence that binds to the cancer associated antigen polypeptide can be determined. One can repeat the procedure using a biased library containing inserts containing part or all of the minimal linear portion plus one or more additional degenerate residues upstream or downstream thereof.

15 Yeast two-hybrid screening methods also may be used to identify polypeptides that bind to the cancer associated antigen polypeptides. Thus, the cancer associated antigen polypeptides of the invention, or a fragment thereof, can be used to screen peptide libraries, including phage display libraries, to identify and select peptide binding partners of the cancer associated antigen polypeptides of the invention. Such molecules can be used, as described, for  
20 screening assays, for purification protocols, for interfering directly with the functioning of cancer associated antigen and for other purposes that will be apparent to those of ordinary skill in the art.

As detailed herein, the foregoing antibodies and other binding molecules may be used for example to identify tissues expressing protein or to purify protein. Antibodies also may be  
25 coupled to specific diagnostic labeling agents for imaging of cells and tissues that express cancer associated antigens or to therapeutically useful agents according to standard coupling procedures. Diagnostic agents include, but are not limited to, barium sulfate, iocetamic acid, iopanoic acid, ipodate calcium, diatrizoate sodium, diatrizoate meglumine, metrizamide, tyropanoate sodium and radiodiagnostics including positron emitters such as fluorine-18 and  
30 carbon-11, gamma emitters such as iodine-123, technitium-99m, iodine-131 and indium-111, nuclides for nuclear magnetic resonance such as fluorine and gadolinium. Other diagnostic agents useful in the invention will be apparent to one of ordinary skill in the art. As used

herein, "therapeutically useful agents" include any therapeutic molecule which desirably is targeted selectively to a cell expressing one of the cancer antigens disclosed herein, including antineoplastic agents, radioiodinated compounds, toxins, other cytostatic or cytolytic drugs, and so forth. Antineoplastic therapeutics are well known and include: aminoglutethimide, azathioprine, bleomycin sulfate, busulfan, carmustine, chlorambucil, cisplatin, cyclophosphamide, cyclosporine, cytarabidine, dacarbazine, dactinomycin, daunorubicin, doxorubicin, taxol, etoposide, fluorouracil, interferon- $\alpha$ , lomustine, mercaptopurine, methotrexate, mitotane, procarbazine HCl, thioguanine, vinblastine sulfate and vincristine sulfate. Additional antineoplastic agents include those disclosed in Chapter 52, Antineoplastic Agents (Paul Calabresi and Bruce A. Chabner), and the introduction thereto, 1202-1263, of Goodman and Gilman's "The Pharmacological Basis of Therapeutics", Eighth Edition, 1990, McGraw-Hill, Inc. (Health Professions Division). Toxins can be proteins such as, for example, pokeweed anti-viral protein, cholera toxin, pertussis toxin, ricin, gelonin, abrin, diphtheria exotoxin, or *Pseudomonas* exotoxin. Toxin moieties can also be high energy-emitting radionuclides such as cobalt-60.

In the foregoing methods, antibodies prepared according to the invention also preferably are specific for the cancer associated antigen/MHC complexes described herein.

When "disorder" is used herein, it refers to any pathological condition where the cancer associated antigens are expressed. An example of such a disorder is cancer, with seminomas, melanomas, teratomas, gliomas, colorectal, ovarian and lung cancers as particular examples.

Samples of tissue and/or cells for use in the various methods described herein can be obtained through standard methods such as tissue biopsy, including punch biopsy and cell scraping, and collection of blood or other bodily fluids by aspiration or other methods.

In certain embodiments of the invention, an immunoreactive cell sample is removed from a subject. By "immunoreactive cell" is meant a cell which can mature into an immune cell (such as a B cell, a helper T cell, or a cytolytic T cell) upon appropriate stimulation. Thus immunoreactive cells include CD34<sup>+</sup> hematopoietic stem cells, immature T cells and immature B cells. When it is desired to produce cytolytic T cells which recognize a cancer associated antigen, the immunoreactive cell is contacted with a cell which expresses a cancer associated antigen under conditions favoring production, differentiation and/or selection of cytolytic T cells; the differentiation of the T cell precursor into a cytolytic T cell upon

exposure to antigen is similar to clonal selection of the immune system.

Some therapeutic approaches based upon the disclosure are premised on a response by a subject's immune system, leading to lysis of antigen presenting cells, such as cancer cells which present one or more cancer associated antigens. One such approach is the administration of autologous CTLs specific to a cancer associated antigen/MHC complex to a subject with abnormal cells of the phenotype at issue. It is within the ability of one of ordinary skill in the art to develop such CTLs *in vitro*. An example of a method for T cell differentiation is presented in International Application number PCT/US96/05607. Generally, a sample of cells taken from a subject, such as blood cells, are contacted with a cell presenting the complex and capable of provoking CTLs to proliferate (e.g., dendritic cells). The target cell can be a transfectant, such as a COS cell. These transfectants present the desired complex of their surface and, when combined with a CTL of interest, stimulate its proliferation. COS cells are widely available, as are other suitable host cells. Specific production of CTL clones is well known in the art. The clonally expanded autologous CTLs then are administered to the subject.

Another method for selecting antigen-specific CTL clones has recently been described (Altman et al., *Science* 274:94-96, 1996; Dunbar et al., *Curr. Biol.* 8:413-416, 1998), in which fluorogenic tetramers of MHC class I molecule/peptide complexes are used to detect specific CTL clones. Briefly, soluble MHC class I molecules are folded *in vitro* in the presence of  $\beta_2$ -microglobulin and a peptide antigen which binds the class I molecule. After purification, the MHC/peptide complex is purified and labeled with biotin. Tetramers are formed by mixing the biotinylated peptide-MHC complex with labeled avidin (e.g. phycoerythrin) at a molar ratio or 4:1. Tetramers are then contacted with a source of CTLs such as peripheral blood or lymph node. The tetramers bind CTLs which recognize the peptide antigen/MHC class I complex. Cells bound by the tetramers can be sorted by fluorescence activated cell sorting to isolate the reactive CTLs. The isolated CTLs then can be expanded *in vitro* for use as described herein.

To detail a therapeutic methodology, referred to as adoptive transfer (Greenberg, *J. Immunol.* 136(5): 1917, 1986; Riddell et al., *Science* 257: 238, 1992; Lynch et al, *Eur. J. Immunol.* 21: 1403-1410, 1991; Kast et al., *Cell* 59: 603-614, 1989), cells presenting the desired complex (e.g., dendritic cells) are combined with CTLs leading to proliferation of the CTLs specific thereto. The proliferated CTLs are then administered to a subject with a

cellular abnormality which is characterized by certain of the abnormal cells presenting the particular complex. The CTLs then lyse the abnormal cells, thereby achieving the desired therapeutic goal.

The foregoing therapy assumes that at least some of the subject's abnormal cells present the relevant HLA/cancer associated antigen complex. This can be determined very easily, as the art is very familiar with methods for identifying cells which present a particular HLA molecule, as well as how to identify cells expressing DNA of the pertinent sequences, in this case a cancer associated antigen sequence. Once cells presenting the relevant complex are identified via the foregoing screening methodology, they can be combined with a sample from a patient, where the sample contains CTLs. If the complex presenting cells are lysed by the mixed CTL sample, then it can be assumed that a cancer associated antigen is being presented, and the subject is an appropriate candidate for the therapeutic approaches set forth *supra*.

Adoptive transfer is not the only form of therapy that is available in accordance with the invention. CTLs can also be provoked *in vivo*, using a number of approaches. One approach is the use of non-proliferative cells expressing the complex. The cells used in this approach may be those that normally express the complex, such as irradiated tumor cells or cells transfected with one or both of the genes necessary for presentation of the complex (i.e. the antigenic peptide and the presenting HLA molecule). Chen et al. (*Proc. Natl. Acad. Sci. USA* 88: 110-114, 1991) exemplifies this approach, showing the use of transfected cells expressing HPVE7 peptides in a therapeutic regime. Various cell types may be used. Similarly, vectors carrying one or both of the genes of interest may be used. Viral or bacterial vectors are especially preferred. For example, nucleic acids which encode a cancer associated antigen polypeptide or peptide may be operably linked to promoter and enhancer sequences which direct expression of the cancer associated antigen polypeptide or peptide in certain tissues or cell types. The nucleic acid may be incorporated into an expression vector. Expression vectors may be unmodified extrachromosomal nucleic acids, plasmids or viral genomes constructed or modified to enable insertion of exogenous nucleic acids, such as those encoding cancer associated antigen, as described elsewhere herein. Nucleic acids encoding a cancer associated antigen also may be inserted into a retroviral genome, thereby facilitating integration of the nucleic acid into the genome of the target tissue or cell type. In these systems, the gene of interest is carried by a microorganism, e.g., a Vaccinia virus, pox virus, herpes simplex virus, retrovirus or adenovirus, and the materials de facto "infect" host cells.

The cells which result present the complex of interest, and are recognized by autologous CTLs, which then proliferate.

A similar effect can be achieved by combining the cancer associated antigen or a stimulatory fragment thereof with an adjuvant to facilitate incorporation into antigen presenting cells *in vivo*. The cancer associated antigen polypeptide is processed to yield the peptide partner of the HLA molecule while a cancer associated antigen peptide may be presented without the need for further processing. Generally, subjects can receive an intradermal injection of an effective amount of the cancer associated antigen. Initial doses can be followed by booster doses, following immunization protocols standard in the art. Preferred cancer associated antigens include those found to react with allogeneic cancer antisera, shown in the examples below.

The invention involves the use of various materials disclosed herein to "immunize" subjects or as "vaccines". As used herein, "immunization" or "vaccination" means increasing or activating an immune response against an antigen. It does not require elimination or eradication of a condition but rather contemplates the clinically favorable enhancement of an immune response toward an antigen. Generally accepted animal models can be used for testing of immunization against cancer using a cancer associated antigen nucleic acid. For example, human cancer cells can be introduced into a mouse to create a tumor, and one or more cancer associated antigen nucleic acids can be delivered by the methods described herein. The effect on the cancer cells (e.g., reduction of tumor size) can be assessed as a measure of the effectiveness of the cancer associated antigen nucleic acid immunization. Of course, testing of the foregoing animal model using more conventional methods for immunization include the administration of one or more cancer associated antigen polypeptides or peptides derived therefrom, optionally combined with one or more adjuvants and/or cytokines to boost the immune response. Methods for immunization, including formulation of a vaccine composition and selection of doses, route of administration and the schedule of administration (e.g. primary and one or more booster doses), are well known in the art. The tests also can be performed in humans, where the end point is to test for the presence of enhanced levels of circulating CTLs against cells bearing the antigen, to test for levels of circulating antibodies against the antigen, to test for the presence of cells expressing the antigen and so forth.

As part of the immunization compositions, one or more cancer associated antigens or

stimulatory fragments thereof are administered with one or more adjuvants to induce an immune response or to increase an immune response. An adjuvant is a substance incorporated into or administered with antigen which potentiates the immune response. Adjuvants may enhance the immunological response by providing a reservoir of antigen (extracellularly or within macrophages), activating macrophages and stimulating specific sets of lymphocytes. Adjuvants of many kinds are well known in the art. Specific examples of adjuvants include monophosphoryl lipid A (MPL, SmithKline Beecham), a congener obtained after purification and acid hydrolysis of *Salmonella minnesota* Re 595 lipopolysaccharide; saponins including QS21 (SmithKline Beecham), a pure QA-21 saponin purified from *Quillja saponaria* extract; DQS21, described in PCT application WO96/33739 (SmithKline Beecham); QS-7, QS-17, QS-18, and QS-L1 (So et al., *Mol. Cells* 7:178-186, 1997); incomplete Freund's adjuvant; complete Freund's adjuvant; vitamin E; montanide; alum; CpG oligonucleotides (see e.g. Kreig et al., *Nature* 374:546-9, 1995); and various water-in-oil emulsions prepared from biodegradable oils such as squalene and/or tocopherol. Preferably, the peptides are administered mixed with a combination of DQS21/MPL. The ratio of DQS21 to MPL typically will be about 1:10 to 10:1, preferably about 1:5 to 5:1 and more preferably about 1:1. Typically for human administration, DQS21 and MPL will be present in a vaccine formulation in the range of about 1 µg to about 100 µg. Other adjuvants are known in the art and can be used in the invention (see, e.g. Goding, *Monoclonal Antibodies: Principles and Practice*, 2nd Ed., 1986). Methods for the preparation of mixtures or emulsions of peptide and adjuvant are well known to those of skill in the art of vaccination.

Other agents which stimulate the immune response of the subject can also be administered to the subject. For example, other cytokines are also useful in vaccination protocols as a result of their lymphocyte regulatory properties. Many other cytokines useful for such purposes will be known to one of ordinary skill in the art, including interleukin-12 (IL-12) which has been shown to enhance the protective effects of vaccines (see, e.g., *Science* 268: 1432-1434, 1995), GM-CSF and IL-18. Thus cytokines can be administered in conjunction with antigens and adjuvants to increase the immune response to the antigens.

There are a number of immune response potentiating compounds that can be used in vaccination protocols. These include costimulatory molecules provided in either protein or nucleic acid form. Such costimulatory molecules include the B7-1 and B7-2 (CD80 and CD86 respectively) molecules which are expressed on dendritic cells (DC) and interact with the

CD28 molecule expressed on the T cell. This interaction provides costimulation (signal 2) to an antigen/MHC/TCR stimulated (signal 1) T cell, increasing T cell proliferation and effector function. B7 also interacts with CTLA4 (CD152) on T cells and studies involving CTLA4 and B7 ligands indicate that the B7-CTLA4 interaction can enhance antitumor immunity and CTL proliferation (Zheng P., et al. *Proc. Natl. Acad. Sci. USA* 95 (11):6284-6289 (1998)).

B7 typically is not expressed on tumor cells so they are not efficient antigen presenting cells (APCs) for T cells. Induction of B7 expression would enable the tumor cells to stimulate more efficiently CTL proliferation and effector function. A combination of B7/IL-6/IL-12 costimulation has been shown to induce IFN-gamma and a Th1 cytokine profile in the T cell population leading to further enhanced T cell activity (Gajewski et al., *J. Immunol.*, 154:5637-5648 (1995)). Tumor cell transfection with B7 has been discussed in relation to *in vitro* CTL expansion for adoptive transfer immunotherapy by Wang et al., (*J. Immunol.*, 19:1-8 (1986)). Other delivery mechanisms for the B7 molecule would include nucleic acid (naked DNA) immunization (Kim J., et al. *Nat Biotechnol.*, 15:7:641-646 (1997)) and recombinant viruses such as adeno and pox (Wendtner et al., *Gene Ther.*, 4:7:726-735 (1997)). These systems are all amenable to the construction and use of expression cassettes for the coexpression of B7 with other molecules of choice such as the antigens or fragment(s) of antigens discussed herein (including polytopes) or cytokines. These delivery systems can be used for induction of the appropriate molecules *in vitro* and for *in vivo* vaccination situations. The use of anti-CD28 antibodies to directly stimulate T cells *in vitro* and *in vivo* could also be considered. Similarly, the inducible co-stimulatory molecule ICOS which induces T cell responses to foreign antigen could be modulated, for example, by use of anti-ICOS antibodies (Hutloff et al., *Nature* 397:263-266, 1999).

Lymphocyte function associated antigen-3 (LFA-3) is expressed on APCs and some tumor cells and interacts with CD2 expressed on T cells. This interaction induces T cell IL-2 and IFN-gamma production and can thus complement but not substitute, the B7/CD28 costimulatory interaction (Parra et al., *J. Immunol.*, 158:637-642 (1997), Fenton et al., *J. Immunother.*, 21:2:95-108 (1998)).

Lymphocyte function associated antigen-1 (LFA-1) is expressed on leukocytes and interacts with ICAM-1 expressed on APCs and some tumor cells. This interaction induces T cell IL-2 and IFN-gamma production and can thus complement but not substitute, the B7/CD28 costimulatory interaction (Fenton et al., *J. Immunother.*, 21:2:95-108 (1998)). LFA-



1 is thus a further example of a costimulatory molecule that could be provided in a vaccination protocol in the various ways discussed above for B7.

Complete CTL activation and effector function requires Th cell help through the interaction between the Th cell CD40L (CD40 ligand) molecule and the CD40 molecule expressed by DCs (Ridge et al., *Nature*, 393:474 (1998), Bennett et al., *Nature*, 393:478 (1998), Schoenberger et al., *Nature*, 393:480 (1998)). This mechanism of this costimulatory signal is likely to involve upregulation of B7 and associated IL-6/IL-12 production by the DC (APC). The CD40-CD40L interaction thus complements the signal 1 (antigen/MHC-TCR) and signal 2 (B7-CD28) interactions.

The use of anti-CD40 antibodies to stimulate DC cells directly would be expected to enhance a response to tumor antigens which are normally encountered outside of a inflammatory context or are presented by non-professional APCs (tumor cells). In these situations Th help and B7 costimulation signals are not provided. This mechanism might be used in the context of antigen pulsed DC based therapies or in situations where Th epitopes have not been defined within known TRA precursors. Other methods for inducing maturation of dendritic cells, e.g., by increasing CD40-CD40L interaction, or by contacting DCs with CpG-containing oligodeoxynucleotides or stimulatory sugar moieties from extracellular matrix, are known in the art.

A cancer associated antigen polypeptide, or a fragment thereof, also can be used to isolate their native binding partners. Isolation of such binding partners may be performed according to well-known methods. For example, isolated cancer associated antigen polypeptides can be attached to a substrate (e.g., chromatographic media, such as polystyrene beads, or a filter), and then a solution suspected of containing the binding partner may be applied to the substrate. If a binding partner which can interact with cancer associated antigen polypeptides is present in the solution, then it will bind to the substrate-bound cancer associated antigen polypeptide. The binding partner then may be isolated.

It will also be recognized that the invention embraces the use of the cancer associated antigen cDNA sequences in expression vectors, as well as to transfect host cells and cell lines, be these prokaryotic (e.g., *E. coli*), or eukaryotic (e.g., dendritic cells, B cells, CHO cells, COS cells, yeast expression systems and recombinant baculovirus expression in insect cells). Especially useful are mammalian cells such as human, mouse, hamster, pig, goat, primate, etc. They may be of a wide variety of tissue types, and include primary cells and cell lines.

Specific examples include keratinocytes, peripheral blood leukocytes, bone marrow stem cells and embryonic stem cells. The expression vectors require that the pertinent sequence, i.e., those nucleic acids described *supra*, be operably linked to a promoter.

The invention also contemplates delivery of nucleic acids, polypeptides or peptides for vaccination. Delivery of polypeptides and peptides can be accomplished according to standard vaccination protocols which are well known in the art. In another embodiment, the delivery of nucleic acid is accomplished by *ex vivo* methods, i.e. by removing a cell from a subject, genetically engineering the cell to include a cancer associated antigen, and reintroducing the engineered cell into the subject. One example of such a procedure is outlined in U.S. Patent 5,399,346 and in exhibits submitted in the file history of that patent, all of which are publicly available documents. In general, it involves introduction *in vitro* of a functional copy of a gene into a cell(s) of a subject, and returning the genetically engineered cell(s) to the subject. The functional copy of the gene is under operable control of regulatory elements which permit expression of the gene in the genetically engineered cell(s). Numerous transfection and transduction techniques as well as appropriate expression vectors are well known to those of ordinary skill in the art. some of which are described in PCT application WO95/00654. *In vivo* nucleic acid delivery using vectors such as viruses and targeted liposomes also is contemplated according to the invention.

In preferred embodiments, a virus vector for delivering a nucleic acid encoding a cancer associated antigen is selected from the group consisting of adenoviruses, adeno-associated viruses, poxviruses including vaccinia viruses and attenuated poxviruses, Semliki Forest virus, Venezuelan equine encephalitis virus, retroviruses, Sindbis virus, and Ty virus-like particle. Examples of viruses and virus-like particles which have been used to deliver exogenous nucleic acids include: replication-defective adenoviruses (e.g., Xiang et al., *Virology* 219:220-227, 1996; Eloit et al., *J. Virol.* 71:5375-5381, 1997; Chengalvala et al., *Vaccine* 15:335-339, 1997), a modified retrovirus (Townsend et al., *J. Virol.* 71:3365-3374, 1997), a nonreplicating retrovirus (Irwin et al., *J. Virol.* 68:5036-5044, 1994), a replication defective Semliki Forest virus (Zhao et al., *Proc. Natl. Acad. Sci. USA* 92:3009-3013, 1995), canarypox virus and highly attenuated vaccinia virus derivative (Paoletti, *Proc. Natl. Acad. Sci. USA* 93:11349-11353, 1996), non-replicative vaccinia virus (Moss, *Proc. Natl. Acad. Sci. USA* 93:11341-11348, 1996), replicative vaccinia virus (Moss, *Dev. Biol. Stand.* 82:55-63, 1994), Venezuelan equine encephalitis virus (Davis et al., *J. Virol.* 70:3781-3787, 1996),

Sindbis virus (Pugachev et al., *Virology* 212:587-594, 1995), and Ty virus-like particle (Allsopp et al., *Eur. J. Immunol* 26:1951-1959, 1996). In preferred embodiments, the virus vector is an adenovirus.

Another preferred virus for certain applications is the adeno-associated virus, a  
5 double-stranded DNA virus. The adeno-associated virus is capable of infecting a wide range of cell types and species and can be engineered to be replication-deficient. It further has advantages, such as heat and lipid solvent stability, high transduction frequencies in cells of diverse lineages, including hematopoietic cells, and lack of superinfection inhibition thus allowing multiple series of transductions. The adeno-associated virus can integrate into  
10 human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function  
15 in an extrachromosomal fashion.

In general, other preferred viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses, the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA.  
20 Adenoviruses and retroviruses have been approved for human gene therapy trials. In general, the retroviruses are replication-deficient (i.e., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high-efficiency transduction of genes *in vivo*. Standard protocols for producing replication-deficient retroviruses (including the  
25 steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell lined with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in Kriegler, M., "Gene Transfer and Expression, A Laboratory Manual," W.H. Freeman Co., New York (1990) and Murry, E.J. Ed. "Methods in  
30 Molecular Biology," vol. 7, Humana Press, Inc., Clifton, New Jersey (1991).

Preferably the foregoing nucleic acid delivery vectors: (1) contain exogenous genetic material that can be transcribed and translated in a mammalian cell and that can induce an

immune response in a host, and (2) contain on a surface a ligand that selectively binds to a receptor on the surface of a target cell, such as a mammalian cell, and thereby gains entry to the target cell.

Various techniques may be employed for introducing nucleic acids of the invention into cells, depending on whether the nucleic acids are introduced *in vitro* or *in vivo* in a host. Such techniques include transfection of nucleic acid-CaPO<sub>4</sub> precipitates, transfection of nucleic acids associated with DEAE, transfection or infection with the foregoing viruses including the nucleic acid of interest, liposome mediated transfection, and the like. For certain uses, it is preferred to target the nucleic acid to particular cells. In such instances, a vehicle used for delivering a nucleic acid of the invention into a cell (e.g., a retrovirus, or other virus; a liposome) can have a targeting molecule attached thereto. For example, a molecule such as an antibody specific for a surface membrane protein on the target cell or a ligand for a receptor on the target cell can be bound to or incorporated within the nucleic acid delivery vehicle. Preferred antibodies include antibodies which selectively bind a cancer associated antigen, alone or as a complex with a MHC molecule. Especially preferred are monoclonal antibodies. Where liposomes are employed to deliver the nucleic acids of the invention, proteins which bind to a surface membrane protein associated with endocytosis may be incorporated into the liposome formulation for targeting and/or to facilitate uptake. Such proteins include capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half life, and the like. Polymeric delivery systems also have been used successfully to deliver nucleic acids into cells, as is known by those skilled in the art. Such systems even permit oral delivery of nucleic acids.

When administered, the therapeutic compositions of the present invention can be administered in pharmaceutically acceptable preparations. Such preparations may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, supplementary immune potentiating agents such as adjuvants and cytokines and optionally other therapeutic agents.

The therapeutics of the invention can be administered by any conventional route, including injection or by gradual infusion over time. The administration may, for example, be oral, intravenous, intraperitoneal, intramuscular, intracavity, subcutaneous, or transdermal. When antibodies are used therapeutically, a preferred route of administration is by pulmonary

aerosol. Techniques for preparing aerosol delivery systems containing antibodies are well known to those of skill in the art. Generally, such systems should utilize components which will not significantly impair the biological properties of the antibodies, such as the paratope binding capacity (see, for example, Sciarra and Cutie, "Aerosols," in Remington's

5 Pharmaceutical Sciences, 18th edition, 1990, pp 1694-1712; incorporated by reference).

Those of skill in the art can readily determine the various parameters and conditions for producing antibody aerosols without resort to undue experimentation. When using antisense preparations of the invention, slow intravenous administration is preferred.

The compositions of the invention are administered in effective amounts. An  
10 "effective amount" is that amount of a cancer associated antigen composition that alone, or together with further doses, produces the desired response, e.g. increases an immune response to the cancer associated antigen. In the case of treating a particular disease or condition characterized by expression of one or more cancer associated antigens, such as seminoma, the desired response is inhibiting the progression of the disease. This may involve only slowing  
15 the progression of the disease temporarily, although more preferably, it involves halting the progression of the disease permanently. This can be monitored by routine methods or can be monitored according to diagnostic methods of the invention discussed herein. The desired response to treatment of the disease or condition also can be delaying the onset or even preventing the onset of the disease or condition.

20 Such amounts will depend, of course, on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be  
25 addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

30 The pharmaceutical compositions used in the foregoing methods preferably are sterile and contain an effective amount of cancer associated antigen or nucleic acid encoding cancer associated antigen for producing the desired response in a unit of weight or volume suitable

for administration to a patient. The response can, for example, be measured by determining the immune response following administration of the cancer associated antigen composition via a reporter system by measuring downstream effects such as gene expression, or by measuring the physiological effects of the cancer associated antigen composition, such as regression of a tumor or decrease of disease symptoms. Other assays will be known to one of ordinary skill in the art and can be employed for measuring the level of the response.

The doses of cancer associated antigen compositions (e.g., polypeptide, peptide, antibody, cell or nucleic acid) administered to a subject can be chosen in accordance with different parameters, in particular in accordance with the mode of administration used and the state of the subject. Other factors include the desired period of treatment. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits.

In general, for treatments for eliciting or increasing an immune response, doses of cancer associated antigen are formulated and administered in doses between 1 ng and 1 mg, and preferably between 10 ng and 100  $\mu$ g, according to any standard procedure in the art. Where nucleic acids encoding cancer associated antigen of variants thereof are employed, doses of between 1 ng and 0.1 mg generally will be formulated and administered according to standard procedures. Other protocols for the administration of cancer associated antigen compositions will be known to one of ordinary skill in the art, in which the dose amount, schedule of injections, sites of injections, mode of administration (e.g., intra-tumoral) and the like vary from the foregoing. Administration of cancer associated antigen compositions to mammals other than humans, e.g. for testing purposes or veterinary therapeutic purposes, is carried out under substantially the same conditions as described above.

Where cancer associated antigen peptides are used for vaccination, modes of administration which effectively deliver the cancer associated antigen and adjuvant, such that an immune response to the antigen is increased, can be used. For administration of a cancer associated antigen peptide in adjuvant, preferred methods include intradermal, intravenous, intramuscular and subcutaneous administration. Although these are preferred embodiments, the invention is not limited by the particular modes of administration disclosed herein.

Standard references in the art (e.g., *Remington's Pharmaceutical Sciences*, 18th edition, 1990) provide modes of administration and formulations for delivery of immunogens with adjuvant

or in a non-adjuvant carrier.

When administered, the pharmaceutical preparations of the invention are applied in pharmaceutically-acceptable amounts and in pharmaceutically-acceptable compositions. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. Such preparations may routinely contain salts, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically-acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic, and the like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

A cancer associated antigen composition may be combined, if desired, with a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid fillers, diluents or encapsulating substances which are suitable for administration into a human. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the molecules of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

The pharmaceutical compositions may contain suitable buffering agents, including: acetic acid in a salt; citric acid in a salt; boric acid in a salt; and phosphoric acid in a salt.

The pharmaceutical compositions also may contain, optionally, suitable preservatives, such as: benzalkonium chloride; chlorobutanol; parabens and thimerosal.

The pharmaceutical compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. All methods include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely

divided solid carrier, or both, and then, if necessary, shaping the product.

Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, lozenges, each containing a predetermined amount of the active compound. Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir or an emulsion.

Compositions suitable for parenteral administration conveniently comprise a sterile aqueous or non-aqueous preparation of cancer associated antigen polypeptides or nucleic acids, which is preferably isotonic with the blood of the recipient. This preparation may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono-or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables. Carrier formulation suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA.

## Examples

### **Materials and Methods**

*Sera, tissues and cell lines.* Sera and tumor tissues were obtained during routine diagnostic or therapeutic procedures. Sera were stored at -80°C until use. Normal tissues were collected from autopsies of tumor-free patients. The study had been approved of by the local ethical review board ("Ethikkommission der Ärztekammer des Saarlandes").

*Construction of cDNA expression libraries.* The construction of the cDNA-expression library enriched for testis-specific transcripts has been described elsewhere (Türeci et al., *Proc. Natl. Acad. Sci. USA* 95:5211-5216, 1998). In brief, based on a previously described suppression subtractive hybridization PCR protocol (Diatchenko et al., *Proc. Natl. Acad. Sci., USA*, 93:6025-6030, 1996), a pool of testis-specific cDNA fragments was prepared using testis mRNA as a tester probe, while the driver probe was represented by a pool of mRNAs



derived from 10 healthy tissues (colon, stomach, brain, resting and activated peripheral blood mononuclear cells (PBMC), skeletal muscle, liver, kidney, lung and skin). The fraction of testis-specific cDNA produced by subtraction was used to capture their long counterparts from a conventional cDNA phagemid library constructed from the original testis mRNA. For capturing, single-stranded pBK-CMV phagemid DNA (Stratagene, Heidelberg, Germany) was extracted after *in vivo* mass excision of the conventional phage expression library using standard protocols and hybridized to the testis cDNA derived from the suppression PCR, the latter being immobilized on nitrocellulose membranes (Schleicher & Schüll, Heidelberg, Germany).

After hybridization the nitrocellulose membranes were washed and phagemid DNA bound to immobilized cDNAs were eluted. Double-stranded cDNA inserts were synthesized using thermostable polymerase from *Pyrococcus furiosus* (Stratagene) and flanking vector-specific primers (pBK Reverse and Universe). Synthesized cDNAs were cut by restriction enzyme digestion and religated into pre-cut dephosphorylated lambda ZAP Express vector. The ligation product was packaged into lambda phage particles and used for transfection and library amplification.

*Immunoscreening of transfectants.* Serum (diluted 1:500) from a 31-year old seminoma patient with seminoma (pT2N0M0) was used to immunoscreen for clones reactive with IgG antibodies as described in detail previously (Sahin, et al., *Proc. Natl. Acad. Sci. USA*, 92:11810-11813, 1995; Türeci et al., *Cancer Res.* 56:4766-4772, 1996).

*Sequence analysis of identified antigens.* Positive clones were subcloned to monoclonality and subjected to *in vivo* excision of pBK-CMV phagemids. The nucleotide sequence of cDNA inserts was determined using EXCEL cycle sequencing kit (Epicentre) on a Li-COR sequencer. Sequencing was performed according to the manufacturers' instructions starting with the vector specific primers and designing transcript-specific ones as the sequencing proceeded. Sequence alignments were performed with DNASIS (Pharmacia Biotech) and BLAST and software on EMBL, Genbank and PROSITE databases.

*Northern blot analysis.* Northern blots were performed with RNA extracted from tumors and normal tissues using standard protocols. Integrity of RNA was checked by

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electrophoresis in formalin/MOPS-gels. Gels containing 10 µg RNA per lane were blotted onto nylon membranes. After prehybridization the members were incubated with transcript-specific <sup>32</sup>P-labeled cDNA probes overnight at 65°C in hybridization solution (ExpressHyb, Clontech, Heidelberg, Germany). The membranes were then washed at progressively higher stringency, with the final wash in 1 x SSC and 0.2% SDS at 65°C. Autoradiography was conducted at -70°C for up to 7 days using Kodak X-OMAT-AR film and intensifying screen.

*Reverse transcription PCR.* Total cellular RNA was extracted and primed with random hexamer and dT(18) oligonucleotides and reverse-transcribed with Superscript II enzyme (Gibco/BRL Life Technologies, Gaithersburg, MD). cDNA thus obtained was tested for integrity by amplification of β-actin transcripts in a 30-cycle PCR reaction. Presence of HOM-TES-85 transcripts was checked with two sets of specific primer pairs. Amplification with 250-S (5' -gga gag gct act caa gat gca gaa gc-3'; SEQ ID NO:1) and 1480-AS (5' -gtt cag ctg ccc aaa gat aca tct acc-3'; SEQ ID NO:2) yielded a 1230bp fragment, whereas 250-S and 865-AS (5' -ctg agt gac tat gag atc tct ctg agt -3'; SEQ ID NO:3) resulted in two fragments (615bp and 429bp) due to the annealing of the 3' -oligonucleotide to a repetitive sequence in the HOM-TES-85 cDNA. Both primers did not amplify fragments from genomic DNA, confirming that they hybridized to different exons. Amplified cDNA products were checked by electrophoresis.

*Southern blot analysis.* Southern blot analysis was performed according to standard protocols (Türeci et al., *Proc. Natl. Acad. Sci. USA* 95:5211-5216, 1998) with digested DNA extracted from peripheral blood lymphocytes from two normal controls. Equal loading of samples was checked by staining with ethidium bromide and visualization of DNA under UV light. Hybridization with a [<sup>32</sup>P]dCTP radiolabelled HOM-TES-85 probe comprising the entire insert of the primary clone was carried out in ExpressHyb buffer at 65°C. Washes and autoradiography were performed as described for Northern blot analysis.

*Screening of sera.* For the detection of antibodies in allogeneic sera from healthy controls and other tumor patients, phages from positive clone HOM-TES-85 were mixed with non-reactive phages of the cDNA library as internal negative controls at a ratio of 1:10 and used to transfect bacteria. 1:200 diluted *E. coli*-absorbed sera from allogeneic patients and

healthy controls were tested on the presence of high-titered HOM-TES-85 specific IgG with the described immunoscreening assay.

**Example 1: Detection of reactive clones and preliminary characterization.**

- 5 Approximately 200,000 clones were screened with the 1:200 diluted serum from a seminoma patient. Nine primary positive clones were found which represented 6 different transcripts (Table 1). As described elsewhere, the initial characterization for each antigen was performed by assessing the nucleotide sequence, the expression pattern in different healthy and neoplastic tissues as well as reactivity with sera from healthy controls and tumor patients.
- 10 Sequence homologies were determined with the BLAST program in Genbank, EMBL and Swissprot databases; accession numbers of homologous database entries are shown in brackets. Sequences of the identified antigen transcripts were submitted to Genbank, and accession numbers are shown in Table 1. The pattern of tissue expression was investigated by specific RT-PCR in a panel of normal cDNAs derived from normal lung, colon, muscle,
- 15 peripheral blood lymphocytes (PBL), liver, kidney, breast, spleen, testis tissues. In selected cases, expression was assessed also by Northern blot. Frequencies of humoral immune responses were determined by phage assay with a panel of sera derived from seminoma and ovarian cancer patients and healthy individuals. Antigens representing the same transcript are referred to in Table 1 as one group.

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**Table 1. Results of basic analysis of antigens identified by immunoscreening of the subtractive cDNA testis expression library.**

Antigen	Genbank Acc. No.	Homology to known genes	Expression in normal tissues	Reactivity with sera
HOM-TES-83 (SEQ ID NO:4)	AF044923	identical to Human hook protein	highly overexpressed in testis shown by Northern blot, faint ubiquitous expression	0/10 healthy 0/14 seminoma
HOM-TES-84 HOM-TES-86 (SEQ ID NO:5)	AF124433	has domains similar to ubiquitin-carboxy-terminal hydrolase genes	n.d.	3/12 healthy
HOM-TES-87 (SEQ ID NO:7)	AF124431	similar to <i>X. laevis</i> mitotic pp43 [U95097]	highly overexpressed in testis shown by Northern blot, expression in normal tissues detectable by RT-PCR	0/10 healthy 1/10 seminoma
HOM-TES-88	AF124434	no homologies	Ubiquitous by RT-PCR,	0/12 healthy

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Antigen	Genbank Acc. No.	H mol gy to kn wn genes	Expression in normal tissues	Reactivity with sera
HOM-TES-94 HOM-TES-95 (SEQ ID NO:8)			not tested by Northern blot	1/11 seminoma
HOM-TES-103 (SEQ ID NO:9)	AF124432	no homologies	Ubiquitous by RT-PCR, not tested by Northern blot	0/12 healthy 1/10 seminoma
HOM-TES-85 (SEQ ID NO:10)	NM_016383; AF124430	no homologies	expression restricted to testis	0/12 healthy 2/10 seminoma 1/7 ovarian cancer

Sequence determination and comparison with databases revealed that the sequence of clone HOM-TES-83 (SEQ ID NO:4) is identical with the putative human counterpart of the *Drosophila* hook-1 protein (Kramer and Phistry, *J. Cell. Biol.*, 133:1205-1215, 1996) known to be involved in the regulation of endocytosis.

Two antigens share sequence similarities with known proteins. HOM-TES-87 (SEQ ID NO:7) has high homologies to the *X. laevis* mitotic protein p43 (Stukenberg et al., *Curr. Biol.* 7:338-348, 1997), most likely being the human counterpart of this proliferation-associated gene. Clones HOM-TES-88, HOM-TES-94 and HOM-TES-95 (SEQ ID NO:8) represent the same transcript with significant homology to the mouse gene PC326 which codes for a product reported to be specific for mouse plasmacytoma (Bergsagel et al., *Oncogene* 7:2059-2064, 1992). Our preliminary RT-PCR expression analysis of this transcript, however, demonstrated a non-restricted expression in normal tissues. Nevertheless, since our first *in silico* cloning attempts revealed that this transcript belongs to a whole family of related sequences and with regard to the specific expression pattern described for the mouse homologue of this gene product, additional studies of the type described herein (e.g., RT-PCR, Northern blot) can determine whether individual members of this family have a more restricted expression pattern.

A search using the BLAST algorithm (Altschul, *J. Mol. Biol.*, 215:403-410, 1990; hosted by the National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/>) revealed no significant nucleotide sequence homologies for clone HOM-TES-84/86 (DNA=SEQ ID NO:5; protein=SEQ ID NO:6, starting at nt370 in reading frame 3). An amino acid pattern search demonstrated that this antigen contains two domains significantly characteristic for ubiquitine-carboxy-terminal hydrolase proteins. Since the gene is devoid of introns, RT-PCR analysis of this transcript was hampered by genomic DNA contamination.

**Example 2: Sequence analysis of HOM-TES-85.**

Sequencing and analysis of the 1747 bp cDNA insert from clone HOM-TES-85 showed an N-terminal methionine complying with the general features of a translation initiation site. This was followed by a 939 bp ORF (nt 34-972 of SEQ ID NO:10) coding for a protein with a predicted molecular weight of 39.5 kDa (SEQ ID NO:11) and by a long 3'-UTR containing a typical polyadenylation signal. Sequence comparison with public databases did not yield any significant identity or homology matches at the nucleic acid or protein levels.

The carboxy terminal part of HOM-TES-85 protein is composed of two long sequentially aligned repeats of homologous heptads (AA 160-288 of SEQ ID NO:11; AA 160-236 and AA 237-288). The second heptad repeat (AA 237-288 of SEQ ID NO:11) contains an unusually long leucine zipper which consists of 8 leucine repeats. Fig. 1 shows a helical wheel diagram of the HOM-TES-85 leucine zipper, in which the residues of the heptad repeats are labeled as a-g.

HOM-TES-85 has putative phosphorylation sites for several kinases, including three sites for cAMP dependent protein kinase near the N-terminus (AA 5-8, AA 56-59 and AA 90-93 of SEQ ID NO:11). Conformational analysis using several algorithms (Garnier et al., *Methods Enzymol.* 266:540-553, 1996; Geourjon and Deleage, *Comput. Appl. Biosci.* 11:681-684, 1995; Deleage and Roux, *Protein Eng.* 1:289-294, 1987) revealed a high probability for  $\alpha$ -helical structures in the leucine zipper region as well as in a N-terminal region with prevailing basic amino acids. Screening for signal sequences and compartmentalization motifs using the pSORT tool (Horton and Nakai, *Intell. Syst. Molec. Biol.* 4: 109-115, 1996) disclosed a 65% affirmative prediction for a nuclear localization of the gene product.

The leucine zipper region of HOM-TES-85 shows an atypical amphipathy, as clusters of hydrophobic residues appear at positions b and c of the helix. As a consequence, the helix exposes hydrophobic surfaces to all directions. Searching the Swissprot-database for other leucine zipper proteins with aliphatic residues at these positions revealed that HOM-TES-85 shares this feature exclusively with the N-myc protooncogene. In the DNA-binding leucine zipper molecules the zipper region is regularly located in the C-terminus and is responsible for mediating specific protein dimerization, which in turn is an essential prerequisite for the DNA-binding activity of an N-terminal basic region. Similarly, the helical N-terminal basic region of HOM-TES-85 may be responsible for interaction with DNA. Together with the

phosphorylation motifs and the predicted nuclear localization HOM-TES-85 may be a regulated tumor-specific DNA-binding protein involved in transcriptional processes in tumor cells.

### 5 **Example 3: Southern Blot analysis of HOM-TES-85.**

Southern blot hybridization of HaeIII-digested high molecular weight genomic DNA from peripheral blood lymphocytes (PBL) and kidney of three different individuals revealed five or six bands with an interindividual polymorphism. Hybridization of a HOM-TES-85 full-length probe to five or six bands suggests either a large single gene or the coexistence of  
10 closely related gene(s).

### **Example 4: Expression spectrum of HOM-TES-85.**

The expression of HOM-TES-85 in normal tissues and tumor specimens was analyzed by Northern blot hybridization and RT-PCR with transcript-specific oligonucleotides on  
15 random hexamer-primed total RNA. The tissues analyzed were normal tissues (lung, spleen, muscle, PBMC, testis, liver, kidney, breast and colon) and tumor specimens (11 melanomas). No expression was detectable in any normal adult tissue, except for testis. Analysis of normal tissues by RT-PCR and Northern blot was repeated using additional tissue types. The results are consistent with the foregoing results and are reported in Table 2.

20

**Table 2. RT-PCR and Northern blot\* analysis of HOM-TES-85/CT-8 expression in normal tissues.**

25	Tissue	Expression (positive / number tested)
	brain	0/4
30	colon	0/2
	lung	0/2
	spleen	0/2
	liver	0/2
	peripheral blood leukocytes	0/2
35	tonsil	0/2
	ovary	0/4
	prostate	0/2
	testis	0/2

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\*Expression analysis was performed by RT-PCR with oligonucleotides specific for HOM-TES-85/CT-8. For selected samples expression was confirmed by Northern blot analysis.

5

Expression in tumor tissues was investigated further by specific RT-PCR of a large panel of tumor-derived first-strand cDNAs, and for selected samples additionally by Northern blot. Primer pairs 293S/865AS and 293S/1480AS were used for RT-PCR and yielded identical results. HOM-TES-85 expression was detected in 7/22 ovarian cancers, in 4/13 seminomas, 2/21 colorectal tumors, 4/12 lung cancers, 8/22 malignant melanoma, 7/20 gliomas, 1/1 teratocarcinoma, and two melanoma cells lines by specific RT-PCR (Table 3). No expression was found in cancers of the prostate, breast, kidney and meningiomas.

10

**Table 3. Expression of HOM-TES-85/CT-8 in malignant tissues.**

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<b>Tumor Type</b>	<b>HOM-TES-85/CT-8 expression (positive / number tested)</b>
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melanoma	8/22
melanoma cell lines	2/2
breast cancer	0/25
colorectal carcinoma	2/21
seminoma	5/13
teratoma	1/1
prostate cancer	0/15
glioma	7/20
meningioma	0/5
lung cancer	4/12
ovarian carcinoma	7/22

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35

With regard to histological subtype, grading and stage, both ovarian cancers derived from coeloma epithelia as well as those derived from the sex cord stroma expressed HOM-TES-85. The majority of ovarian tumors included in the study were of epithelial origin, and five of them expressed HOM-TES-85/CT-8. Two specimens were derived from the sex cord stroma (one Sertoli-Leydig cell-tumor, one moderately differentiated granulosa cell tumor), and both expressed HOM-TES-85/CT-8. The data show that HOM-TES-85/CT-8 expression

40

is not restricted to ovarian cancers of a particular origin.

Probing of positive specimens in Northern blots revealed transcripts of 1.85 kb which is compatible with the cloned full-length sequence together with a poly(A)-tail. The length of exposure times suggested a moderately abundant transcript level in the tumors compared to testis.

HOM-TES-85 fulfills the criteria for CT antigens as its expression is not detectable in normal tissues except for testis (Table 2), but in a varying proportion of different tumor types (Table 3). According to the suggested nomenclature of CTA (Gaugler et al., *J. Exp. Med.*, 179:921-930, 1995) designation of HOM-TES-85 as CT-8 is proposed. While many CTA have been shown to be expressed in melanomas, HOM-TES-85 is potentially of great clinical interest for seminomas, gliomas, lung cancer, and ovarian cancers, where the expression of the CTA known to date is comparatively rare.

#### Example 5: Screening of allogeneic sera for anti-HOM-TES-85 antibodies.

To evaluate the frequency of anti-HOM-TES-85 IgG antibodies, sera of patients and normal controls were screened using the phage assay. One of seven patients with ovarian cancer and one additional patient out of 15 patients with seminoma had a detectable serum reactivity to HOM-TES-85, while sera from controls and patients suffering from other types of cancer were negative (Table 4). All three patients with anti-HOM-TES-85 antibody responses had tumors that expressed HOM-TES-85 as assessed by RT-PCR.

**Table 4. Humoral immune responses to HOM-TES-85/CT-8 as assessed by phage-assay.**

diagnosis of patients	number of positive sera / number tested
seminoma	2/15
ovarian cancer	1/7
breast cancer	0/8
melanoma	0/10
healthy controls	0/20

#### Example 6: Preparation of recombinant cancer associated antigens

To facilitate screening of patients' sera for antibodies reactive with cancer associated antigens, for example by ELISA, recombinant proteins are prepared according to standard



procedures. In one method, the clones encoding cancer associated antigens are subcloned into a baculovirus expression vector, and the recombinant expression vectors are introduced into appropriate insect cells. Baculovirus/insect cloning systems are preferred because post-translational modifications are carried out in the insect cells. Another preferred eukaryotic system is the *Drosophila* Expression System from Invitrogen. Clones which express high amounts of the recombinant protein are selected and used to produce the recombinant proteins. The recombinant proteins are tested for antibody recognition using serum from the patient which was used to isolated the particular clone, or in the case of cancer associated antigens recognized by allogeneic sera, by the sera from any of the patients used to isolate the clones or sera which recognize the clones' gene products.

Alternatively, the cancer associated antigen clones are inserted into a prokaryotic expression vector for production of recombinant proteins in bacteria. Other systems, including yeast expression systems and mammalian cell culture systems also can be used.

#### **Example 7: Preparation of antibodies to cancer associated antigens**

The recombinant cancer associated antigens produced as in Example 6 above are used to generate polyclonal antisera and monoclonal antibodies according to standard procedures. The antisera and antibodies so produced are tested for correct recognition of the cancer associated antigens by using the antisera/antibodies in assays of cell extracts of patients known to express the particular cancer associated antigen (e.g. an ELISA assay). These antibodies can be used for experimental purposes (e.g. localization of the cancer associated antigens, immunoprecipitations, Western blots, etc.) as well as diagnostic purposes (e.g., testing extracts of tissue biopsies, testing for the presence of cancer associated antigens).

#### **Example 8: Expression of cancer associated antigens in cancers of similar and different origin.**

The expression of one or more of the cancer associated antigens is tested in a range of tumor samples to determine which, if any, other malignancies should be diagnosed and/or treated by the methods described herein. Tumor cell lines and tumor samples are tested for expression of cancer associated antigens not already tested (e.g., HOM-TES-84, 86, 88, 94, 95, 103) preferably by RT-PCR and Northern blot procedures as described above. Antibody based assays, such as ELISA and western blot, also can be used to determine protein

expression of all of the cancer associated antigens. A preferred method of testing expression of cancer associated antigens (in other cancers and in additional same type cancer patients) is allogeneic serotyping using a modified SEREX protocol (as described above).

In all of the foregoing, extracts from the tumors of patients who provided sera for the  
5 initial isolation of the cancer associated antigens preferably are used as positive controls. The cells containing recombinant expression vectors described in the Examples above also can be used as positive controls.

The results generated from the foregoing experiments provide panels of multiple cancer associated nucleic acids and/or polypeptides for use in diagnostic (e.g. determining the  
10 existence of cancer, determining the prognosis of a patient undergoing therapy, etc.) and therapeutic methods (e.g., vaccine composition, etc.).

#### **Example 9: HLA typing of patients positive for cancer associated antigens**

To determine which HLA molecules present peptides derived from the cancer  
15 associated antigens disclosed herein, cells of the patients which express the cancer associated antigens are HLA typed. Peripheral blood lymphocytes are taken from the patient and typed for HLA class I or class II, as well as for the particular subtype of class I or class II. Tumor biopsy samples also can be used for typing. HLA typing can be carried out by any of the standard methods in the art of clinical immunology, such as by recognition by specific  
20 monoclonal antibodies, or by HLA allele-specific PCR (e.g. as described in WO97/31126).

#### **Example 10: Characterization of cancer associated antigen peptides presented by MHC class I and class II molecules.**

Antigens which provoke an antibody response in a subject may also provoke a cell-  
25 mediated immune response. Cells process proteins into peptides for presentation on MHC class I or class II molecules on the cell surface for immune surveillance. Peptides presented by certain MHC/HLA molecules generally conform to motifs. These motifs are known in some cases, and can be used to screen the cancer associated antigens for the presence of potential class I and/or class II peptides. Summaries of class I and class II motifs have been  
30 published (e.g., Rammensee et al., *Immunogenetics* 41:178-228, 1995). Based on the results of experiments such as those described above, the HLA types which present the individual cancer associated antigens are known. Motifs of peptides presented by these HLA molecules

thus are preferentially searched.

One also can search for class I and class II motifs using computer algorithms. For example, computer programs for predicting potential CTL epitopes based on known class I motifs has been described (*see, e.g., Parker et al, J. Immunol. 152:163, 1994; D'Amato et al., Human Immunol. 43:13-18, 1995; Drijfhout et al., Human Immunol. 43:1-12, 1995*). HLA binding predictions can conveniently be made using an algorithm available via the Internet on the National Institutes of Health World Wide Web site at URL <http://bimas.dcrt.nih.gov>, for example, using default settings. For example, several predicted HLA binding peptides were identified for HOM-TES-84 and HOM-TES-85 proteins, and are listed in the table below:

**Table 5: Predicted HLA class I binding motifs in HOM-TES-85**

**HOM-TES-84 HLA peptides**

	HLA	Sequence	Position	SEQ ID NO
	A1	KVEPNNYLSI	447 - 456	12
	A1	ELEYNCQMCK	481 - 490	13
20	A1	NNEQVYIPK	525 - 533	14
	A1	NADLQRFQR	600 - 608	15
	A1	VTESTNGFY	708 - 716	16
	A1	MGDPLQAYR	816 - 824	17
	A1	ISDVYDFQK	842 - 850	18
25	A_0201	KLKEALIE TV	21 - 30	19
	A_0201	FQLSNNIRSV	51 - 60	20
	A_0201	QLSNNIRSV	52 - 60	21
	A_0201	RLTLKNNVFL	73 - 82	22
30	A_0201	NVFLFIDKL	79 - 87	23
	A_0201	KLSYRDAKQL	86 - 95	24
	A_0201	KQLNMFLDI	93 - 101	25
	A_0201	SVFESRNML	118 - 126	26
	A_0201	NMLKEIDKT	124 - 132	27
35	A_0201	FMSKSPTHV	149 - 157	28
	A_0201	KLGPSFNTNC	223 - 232	29
	A_0201	NLDETVLAT	237 - 245	30
	A_0201	QLQQGF PNL	282 - 290	31
	A_0201	YMNAVLQSL	295 - 303	32
40	A_0201	AVLQSLFAI	298 - 306	33
	A_0201	SLFAIPSFA	302 - 310	34
	A_0201	ALIMTLTQL	327 - 335	35
	A_0201	IMTLTQLLAL	329 - 338	36
	A_0201	QLLALKDFC	334 - 342	37
45	A_0201	LLALKDFCST	335 - 344	38
	A_0201	ELLGNVKKV	349 - 357	39

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	A_0201	NMQNDAHEFL	368 - 377	40
	A_0201	MQNDAHEFL	369 - 377	41
	A_0201	FLGQCLDQL	376 - 384	42
	A_0201	VVANFEFEL	424 - 432	43
5	A_0201	FEFELQLSLI	428 - 437	44
	A_0201	YLSINLHQET	453 - 462	45
	A_0201	NLHQETKPL	457 - 465	46
	A_0201	QMCKQKSCV	487 - 495	47
	A_0201	CQMCKQKSCV	486 - 495	48
10	A_0201	RLSRVLI IHL	502 - 511	49
	A_0201	WLLVKNNQV	520 - 529	50
	A_0201	LLVKNNQV	521 - 529	51
	A_0201	MISEINSPL	569 - 577	52
	A_0201	KLTSESSDSL	582 - 591	53
15	A_0201	AIGEKELPV	639 - 647	54
	A_0201	SLMDQGDISL	650 - 659	55
	A_0201	VMYEDGGKLI	661 - 670	56
	A_0201	KLISSPDTRL	668 - 677	57
	A_0201	RLVEVHLQEV	676 - 685	58
20	A_0201	GMAEQLQQC	729 - 737	59
	A_0201	SIIDEFLQQA	741 - 750	60
	A_0201	FLQQAPPPGV	746 - 755	61
	A_0201	TLNQSTELRL	767 - 776	62
	A_0201	RLQKADLNHL	775 - 784	63
25	A_0201	LQAYRLISV	820 - 828	64
	A_0201	RLISVVVSHI	824 - 832	65
	A_0201	YIFFYMHNGI	878 - 887	66
	A_0201	YMHNGIFEEL	882 - 891	67
	A_0201	RLPSTQAGV	899 - 907	68
30	A3	KLVVTFKSGK	37 - 46	69
	A3	FLFIDKLSY	81 - 89	70
	A3	FLDIIHQNK	98 - 106	71
	A3	SVFESRNMLK	118 - 127	72
35	A3	FMSKSPTHVK	149 - 158	73
	A3	SLKYIQSNRK	199 - 208	74
	A3	VLATQTLNAK	242 - 251	75
	A3	TLTQLLALK	331 - 339	76
	A3	ALKDFCSTK	337 - 345	77
40	A3	FLGQCLDQLK	376 - 385	78
	A3	KLNATLNTGK	390 - 399	79
	A3	QMHVGSAAATK	409 - 418	80
	A3	SLVLPVEPDK	590 - 599	81
	A3	KMGDPLQAY	815 - 823	82
45	A3	RLHSGYIFFY	873 - 882	83
	A3	GIFEELLRK	886 - 894	84
	A24	SYRDAKQLNM	88 - 97	85
	A24	SYQKMPLFM	142 - 150	86
50	A24	KYKTDSLKYI	194 - 203	87
	A24	CYMAVLQSL	294 - 303	88
	A24	EYIPFEALI	321 - 329	89
	A24	EFLGQCLDQL	375 - 384	90

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	A24	VFVCPVVANF	419 - 428	91
	A24	NFEFELQLSL	427 - 436	92
	A24	TFSRLSRVL	499 - 507	93
	A24	RYSFNNAWL	513 - 521	94
5	A24	VYIPKSLSL	529 - 537	95
	A24	MYEDGGKLI	662 - 670	96
	A24	FYDCKENRI	715 - 723	97
	A24	HYISDVYDF	840 - 848	98
	A24	VYDFQKQAWF	845 - 854	99
10	A24	TYNDLCVSEI	855 - 864	100
	B7	VQRQKEIKL	30 - 38	101
	B7	TVQRQKEIKL	29 - 38	102
	B7	NVFLFIDKL	79 - 87	23
15	B7	SVFESRNML	118 - 126	26
	B7	KPSYQKMPL	140 - 148	103
	B7	SPTHVKKGIL	153 - 162	104
	B7	DVQTNEDIL	176 - 184	105
	B7	SNRKNPSSL	205 - 213	106
20	B7	NPNLDETVL	235 - 243	107
	B7	DPRCNKAQV	266 - 274	108
	B7	VPLDSHSQQL	274 - 283	109
	B7	FPNLGNTCYM	287 - 296	110
	B7	IPSFADDLL	306 - 314	111
25	B7	IPFEALIMTL	323 - 332	112
	B7	VVANFEFEL	424 - 432	43
	B7	EPNNYLSINL	449 - 458	113
	B7	LPLSIQNSL	465 - 473	114
	B7	VARHTFSRL	495 - 503	115
30	B7	CVARHTFSRL	494 - 503	116
	B7	LSRVLIHIL	503 - 511	117
	B7	QVYIPKSLSL	528 - 537	118
	B7	APVGKCEVL	555 - 563	119
	B7	SPLTPSMKL	575 - 583	120
35	B7	DTRLVEVHL	674 - 682	121
	B7	APPPGVRKL	750 - 758	122
	B7	ELRLQKADL	773 - 781	123
	B7	NTRGEAKEL	802 - 810	124
	B7	LLRKAENSRL	891 - 900	125
40	B8	MTKLKEALI	19 - 27	126
	B8	VQRQKEIKL	30 - 38	101
	B8	HCKKRQSHL	64 - 72	127
	B8	DAKQLNMFL	91 - 99	128
45	B8	STKIKRELL	343 - 351	129
	B8	FSRLSRVLII	500 - 509	130
	B8_8mer	MTKLKEAL	19 - 26	131
	B8_8mer	STKIKREL	343 - 350	132
	B8_8mer	MCKQKSCV	488 - 495	133
50	B_3501	KPSYQKMPL	140 - 148	103
	B_3501	SPTHVKKGIL	153 - 162	104
	B_3501	NPNLDETVL	235 - 243	107

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	B_3501	VPLDSHSQQL	274 - 283	109
	B_3501	FPNLTGNTCY	287 - 295	134
	B_3501	IPSFADDLL	306 - 314	111
	B_3501	IPFEALIMTL	323 - 332	112
5	B_3501	CPVVANFEF	422 - 430	135
	B_3501	EPNNYLSINL	449 - 458	113
	B_3501	LPLSIQNSL	465 - 473	114
	B_3501	IPKSLSLSSY	531 - 540	136
	B_3501	APVGKCEVL	555 - 563	119
10	B_3501	SPLTPSMKL	575 - 583	120
	B_3501	VPQHPELQKY	685 - 694	137
	B_3501	APPPGVRKL	750 - 758	122
	B3501_8mer	NPVVPNKKY	188 - 195	138
	B3501_8mer	NPSSLEDL	209 - 216	139
15	B3501_8mer	IPSFADDL	306 - 313	140
	B3501_8mer	VPWEYIPF	318 - 325	141
	B3501_8mer	IPFEALIM	323 - 330	142
	B3501_8mer	KSLSLSSY	533 - 540	143
	B3501_8mer	LPVADSLM	645 - 652	144
20	B3501_8mer	NPGNKNIL	791 - 798	145
	B3501_8mer	DPLQAYRL	818 - 825	146
	B_4403	KEIKLVVTF	34 - 42	147
	B_4403	KEIDKTSFY	127 - 135	148
25	B_4403	EDNPVPNKKY	186 - 195	149
	B_4403	DNPVPNKKY	187 - 195	150
	B_4403	DETVLATQT	239 - 247	151
	B_4403	RELLGNVKKV	348 - 357	152
	B_4403	SEINSPLTPS	571 - 580	153
30	B_4403	KELPVADSL	643 - 651	154
	B_4403	GDISLPVMY	655 - 663	155
	B_4403	YEKTNTFVEF	694 - 703	156
	B_4403	IEESIIDEF	738 - 746	157
35	B_4403	QEARLHSGY	870 - 878	158

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**HOM-TES-85 HLA peptides**


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	HLA	Sequence	Position	SEQ ID NO
<hr/>				
40	A1	SLDDIIYK	29 - 37	159
	A1	NSEEGNHDK	82 - 90	160
45	A_0201	FLDMSLDDI	25 - 33	161
	A_0201	DLIVTQRDL	272 - 280	162
	A_0201	DLIATQRDL	265 - 273	163
			258 - 266	163
			244 - 252	163
50	A_0201	LIVTQRDLV	273 - 281	164
	A_0201	LIATQRDLI	266 - 275	165
			259 - 257	165
			252 - 260	165

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	A_0201	DLIATQKDL	251 - 259	166
	A_0201	DLVDTQSDL	237 - 245	167
	A_0201	IIYKELEGT	34 - 242	168
	A_0201	DLVATERDL	279 - 287	169
5	A_0201	KVNFLDMSL	22 - 30	170
	A_0201	TLSEKVPPN	8 - 16	171
	A_0201	SLDDIIIIYK	29 - 37	159
	A_0201	LVATERDLI	280 - 288	172
	A_0201	VTQORDLVAT	275 - 283	173
10	A_0201	IATQORDLIV	267 - 275	174
	A_0201	SRNHLESL	162 - 170	175
	A_0201	MASFRKLT	1 - 9	176
	A_0201	ATQORDLIAT	261 - 269	177
			247 - 255	177
15	A_0201	ATQKDLIAT	254 - 262	178
	A_0201	IIIIYKELEG	33 - 41	179
	A_0201	DMSLDDIII	27 - 35	180
	A_0201	RKLTLSSEKV	5 - 13	181
	A_0201	ATQORDLIVT	268 - 276	182
20	A_0201	LVDTQSDLI	238 - 246	183
	A_0201	LIATQORDLIV	266 - 275	184
	A_0201	DLIVTQORDLV	272 - 281	185
	A_0201	IIIIYKELEGT	33 - 42	186
	A_0201	SLDDIIIIYKE	29 - 38	187
25	A_0201	DLIATQORDLI	265 - 274	188
			258 - 257	188
			244 - 253	188
	A_0201	DLIATQKDLI	251 - 260	189
	A_0201	FLDMSLDDII	25 - 34	190
30	A_0201	DLVDTQSDLI	237 - 246	191
	A_0201	DLVATERDLI	279 - 288	192
	A_0201	LIATQORDLIA	259 - 268	193
			245 - 254	193
	A_0201	LIATQKDLIA	252 - 261	194
35	A_0201	IVTQORDLVAT	274 - 283	195
	A3	SLDDIIIIYK	29 - 37	159
	A24	GFKSGQHPL	98 - 106	196
40	A24	RYSTGKNTI	303 - 311	197
	B7	KVNFLDMSL	22 - 30	170
	B7	HPSRKKVNFL	17 - 26	198
	B7	RSRNHLESL	161 - 170	199
45	B_3501	HPSRKKVNF	17 - 25	200
	B_3501	MSLDDIIIIY	28 - 36	201
	B_3501	KPSQKPSGF	91 - 99	202
	B_3501	RSRNHLESL	161 - 170	199
50	B3501_8mer	HPLNGQPL	104 - 111	203
	B3501_8mer	RSRSQGDL	231 - 238	204
	B_4403	IEQEKCSNY	112 - 121	205

Methods for determining HLA class II peptides and making substitutions thereto are also known (e.g. Strominger and Wucherpennig (PCT/US96/03182)).

5 **Example 11: Identification of the portion of a cancer associated polypeptide encoding an antigen**

To determine if the cancer associated antigens isolated as described above can provoke a cytolytic T lymphocyte response, the following method is performed for generation of CD8<sup>+</sup> T cells using the predicted HLA peptides described above.

10 Briefly, peripheral blood mononuclear cells (PBMCs) are isolated using ficoll-Hypaque (Pharmacia) from blood of HLA-A201<sup>+</sup> healthy donors (or donors positive for whatever HLA binding peptide is being investigated). Adherent monocytes are separated from non-adherent peripheral blood lymphocytes (PBLs) by plastic adherence via overnight incubation at 37°C on plastic. The non-adherent PBLs are cryopreserved until needed, while  
15 adherent cells are stimulated to differentiate into dendritic cells (DCs) by incubation in AIMV-medium (Gibco), containing 1000 U/ml IL4 and 1000 U/ml GM-CSF for 5 days.

On day 7,  $8 \times 10^5$  DCs are loaded with 50mg/ml exogenously added peptide for 2 hours at 37°C in medium containing 1000 U/ml TNF $\alpha$  and 10,000 U/ml IL1 $\beta$ . The peptide pulsed DCs are then washed twice in excess medium containing no peptide. Autologous PBLs are  
20 thawed and  $4 \times 10^7$  PBLs are coincubated with  $8 \times 10^5$  peptide loaded DCs (ratio 50:1), in medium containing 5ng/ml IL-7 and 20 U/ml IL2. These cultures are then incubated at 37°C.

On days 14, 21 and 28 the lymphocyte cultures are further stimulated with autologous peptide pulsed DC cells as for day 7. Functional assays such as Elispot and cytotoxicity assays are done on days 14, 21 and/or 28. The Elispot assays are performed using  
25 commercially available Elispot-system for IFN- $\gamma$ ; NC plates from Millipore; antibodies from Holzel, following manufacturers instructions. The cytotoxicity assays are performed using a Europium labelling based assay which is analogous to chromium release type assay (Blomberg et al., *J. Immunol Methods* 114:191-195, 1988).

A similar method is performed to determine if the cancer associated antigen contains  
30 one or more HLA class II peptides recognized by T cells. One can search the sequence of the cancer associated antigen polypeptides for HLA class II motifs as described above. In contrast to class I peptides, class II peptides are presented by a limited number of cell types. Thus for



these experiments, dendritic cells or B cell clones which express HLA class II molecules preferably are used.

**Table 6: Sequence homologies listing GenBank accession numbers**

HOM-TES-83 (SEQ ID NO:4)

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HOM-TES-84/86 3' (SEQ ID NO:5)

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HOM-TES-84/86 5' (SEQ ID NO:6)

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- AI043714, AI009641, AI138042, AI137138, AI137227, AI176056, AA859564, AA859963, AA901411, D48239, AA109365, AI045829, AI071234, AA109423, AA957307, AA858599, AI065703, AI071422, AI138012, AI104268, AI100474, AI137107, AA925514, AI137436, AI177791, N28061, AI237589, AA840999, AA997716, AF083278, D47088, L19151, AI102579, AA901058, AI136747, AI237633, AI113047,
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- 35 THC159576, THC136194, THC165367, THC165368.

### Claims

1. A method of diagnosing a disorder characterized by expression of a human cancer associated antigen precursor coded for by a nucleic acid molecule, comprising:

contacting a biological sample isolated from a subject with an agent that specifically  
5 binds to the nucleic acid molecule, an expression product thereof, or a fragment of an expression product thereof complexed with an HLA molecule, wherein the nucleic acid molecule is a NA Group 1 nucleic acid molecule, and

determining the interaction between the agent and the nucleic acid molecule or the expression product as a determination of the disorder.

10 2. The method of claim 1, wherein the agent is selected from the group consisting of

(a) a nucleic acid molecule comprising NA group 1 nucleic acid molecules or a fragment thereof,

(b) a nucleic acid molecule comprising NA group 3 nucleic acid molecules or a  
15 fragment thereof,

(c) a nucleic acid molecule comprising NA group 5 nucleic acid molecules or a fragment thereof,

(d) an antibody that binds to an expression product of NA group 1 nucleic acids,

(e) an antibody that binds to an expression product of NA group 3 nucleic acids,

20 (f) an antibody that binds to an expression product of NA group 5 nucleic acids,

(g) an agent that binds to a complex of an HLA molecule and a fragment of an expression product of a NA group 1 nucleic acid,

(h) an agent that binds to a complex of an HLA molecule and a fragment of an expression product of a NA group 3 nucleic acid, and

25 (i) an agent that binds to a complex of an HLA molecule and a fragment of an expression product of a NA group 5 nucleic acid.

3. The method of claim 1, wherein the disorder is characterized by expression of a plurality of human cancer associated antigen precursors and wherein the agent is a plurality of  
30 agents, each of which is specific for a different human cancer associated antigen precursor, and wherein said plurality of agents is at least 2, at least 3, at least 4, at least 4, at least 6, at least 7, or at least 8, at least 9 or at least 10 such agents.



4. The method of claims 1-3, wherein the agent is specific for a human cancer associated antigen precursor that is a seminoma cancer associated antigen precursor.

5. A method for determining regression, progression or onset of a condition characterized by expression of abnormal levels of a protein encoded by a nucleic acid molecule that is a NA Group 1 molecule, comprising

monitoring a sample, from a patient who has or is suspected of having the condition, for a parameter selected from the group consisting of

- (i) the protein,
  - (ii) a peptide derived from the protein,
  - (iii) an antibody which selectively binds the protein or peptide, and
  - (iv) cytolytic T cells specific for a complex of the peptide derived from the protein and an MHC molecule,
- as a determination of regression, progression or onset of said condition.

6. The method of claim 5, wherein the sample is a body fluid, a body effusion or a tissue.

7. The method of claim 5, wherein the step of monitoring comprises contacting the sample with a detectable agent selected from the group consisting of

- (a) an antibody which selectively binds the protein of (i), or the peptide of (ii),
- (b) a protein or peptide which binds the antibody of (iii), and
- (c) a cell which presents the complex of the peptide and MHC molecule of (iv).

8. The method of claim 7, wherein the antibody, the protein, the peptide or the cell is labeled with a radioactive label or an enzyme.

9. The method of claim 5, comprising assaying the sample for the peptide.

10. The method of claim 5, wherein the nucleic acid molecule is a NA Group 3 molecule.

11. The method of claim 5, wherein the nucleic acid molecule is a NA Group 5 molecule.

12. The method of claim 5, wherein the protein is a plurality of proteins, the parameter is a plurality of parameters, each of the plurality of parameters being specific for a different of the plurality of proteins, at least one of which is a cancer associated protein encoded by a NA Group 1 molecule.

5

13. A pharmaceutical preparation for a human subject comprising  
an agent which when administered to the subject enriches selectively the presence of  
complexes of an HLA molecule and a human cancer associated antigen, and  
a pharmaceutically acceptable carrier, wherein the human cancer associated antigen is  
10 a fragment of a human cancer associated antigen precursor encoded by a nucleic acid  
molecule comprises a NA Group 1 molecule.

15

14. The pharmaceutical preparation of claim 13, wherein the agent comprises a plurality of  
agents, each of which enriches selectively in the subject complexes of an HLA molecule and a  
different human cancer associated antigen, wherein at least one of the human cancer  
associated antigens is encoded by a NA Group 1 molecule.

20

15. The pharmaceutical preparation of claim 14, wherein the plurality is at least two, at  
least three, at least four or at least 5 different such agents.

16. The pharmaceutical preparation of claim 13, wherein the nucleic acid molecule is a  
NA Group 3 nucleic acid molecule.

25

17. The pharmaceutical preparation of claim 13, wherein the agent is selected from the  
group consisting of

(1) an isolated polypeptide comprising the human cancer associated antigen, or a  
functional variant thereof,

(2) an isolated nucleic acid operably linked to a promoter for expressing the isolated  
polypeptide, or functional variant thereof,

30

(3) a host cell expressing the isolated polypeptide, or functional variant thereof, and  
(4) isolated complexes of the polypeptide, or functional variant thereof, and an HLA  
molecule.

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18. The pharmaceutical preparation of claims 13-17, further comprising an adjuvant.

19. The pharmaceutical preparation of claim 13, wherein the agent is a cell expressing an isolated polypeptide comprising the human cancer associated antigen or a functional variant thereof, and wherein the cell is nonproliferative.

20. The pharmaceutical preparation of claim 13, wherein the agent is a cell expressing an isolated polypeptide comprising the human cancer associated antigen or a functional variant thereof, and wherein the cell expresses an HLA molecule that binds the polypeptide.

21. The pharmaceutical preparation of claim 13, wherein the agent is at least two, at least three, at least four or at least five different polypeptides, each coding for a different human cancer associated antigen or functional variant thereof, wherein at least one of the human cancer associated antigens is encoded by a NA Group 1 molecule.

22. The pharmaceutical preparation of claim 13, wherein the agent is a PP Group 2 polypeptide.

23. The pharmaceutical preparation of claim 13, wherein the agent is a PP Group 3 polypeptide or a PP Group 4 polypeptide.

24. The pharmaceutical preparation of claim 20, wherein the cell expresses one or both of the polypeptide and HLA molecule recombinantly.

25. The pharmaceutical preparation of claim 20, wherein the cell is nonproliferative.

26. A composition of matter comprising an isolated agent that binds selectively a PP Group 1 polypeptide.

27. The composition of matter of claim 26, wherein the agent binds selectively a PP Group 2 polypeptide.

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28. The composition of matter of claim 26, wherein the agent binds selectively a PP Group 3 polypeptide.

29. The composition of matter of claim 26, wherein the agent binds selectively a PP Group 4 polypeptide.

30. The composition of matter of claim 26, wherein the agent binds selectively a PP Group 5 polypeptide.

31. The composition of matter of claims 26-30, wherein the agent is a plurality of different agents that bind selectively at least two, at least three, at least four, or at least five different such polypeptides.

32. The composition of matter of claims 26-30, wherein the agent is an antibody.

33. The composition of matter of claim 31, wherein the agent is an antibody.

34. A composition of matter comprising  
a conjugate of the agent of claims 26-30 and a therapeutic or diagnostic agent.

35. A composition of matter comprising  
a conjugate of the agent of claim 31 and a therapeutic or diagnostic agent.

36. The composition of matter of claim 34, wherein the conjugate is of the agent and a therapeutic or diagnostic that is a toxin.

37. A pharmaceutical composition comprising an isolated nucleic acid molecule selected from the group consisting of NA Group 1 molecules and NA Group 2 molecules, and a pharmaceutically acceptable carrier.

38. The pharmaceutical composition of claim 37, wherein the isolated nucleic acid molecule comprises a NA Group 3 or NA Group 4 molecule.

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39. The pharmaceutical composition of claim 37, wherein the isolated nucleic acid molecule comprises at least two isolated nucleic acid molecules coding for two different polypeptides, each polypeptide comprising a different human cancer associated antigen.

5 40. The pharmaceutical composition of claims 37-39 further comprising an expression vector with a promoter operably linked to the isolated nucleic acid molecule.

41. The pharmaceutical composition of claims 37-39 further comprising a host cell recombinantly expressing the isolated nucleic acid molecule.

10 42. A pharmaceutical composition comprising  
an isolated polypeptide comprising a PP Group 1 or a PP Group 2 polypeptide, and  
a pharmaceutically acceptable carrier.

15 43. The pharmaceutical composition of claim 42, wherein the isolated polypeptide comprises a PP Group 3 or a PP Group 4 polypeptide.

44. The pharmaceutical composition of claim 42, wherein the isolated polypeptide comprises at least two different polypeptides, each comprising a different human cancer  
20 associated antigen.

45. The pharmaceutical composition of claims 42-44, further comprising an adjuvant.

46. An isolated nucleic acid molecule comprising a NA Group 3 molecule.

25 47. An isolated nucleic acid molecule comprising a NA Group 4 molecule.

48. An isolated nucleic acid molecule selected from the group consisting of  
(a) a fragment of a nucleic acid molecule having a nucleotide sequence selected from  
30 the group consisting of nucleotide sequences set forth as SEQ ID NOs:4-10, of sufficient  
length to represent a sequence unique within the human genome, and identifying a nucleic  
acid encoding a human cancer associated antigen precursor,

(b) complements of (a),

provided that the fragment includes a sequence of contiguous nucleotides which is not identical to any sequence selected from the sequence group consisting of

(1) sequences having the GenBank accession numbers of Table 6,

5 (2) complements of (1), and

(3) fragments of (1) and (2).

49. The isolated nucleic acid molecule of claim 48, wherein the sequence of contiguous nucleotides is selected from the group consisting of:

- 10 (1) at least two contiguous nucleotides nonidentical to the sequence group,  
(2) at least three contiguous nucleotides nonidentical to the sequence group,  
(3) at least four contiguous nucleotides nonidentical to the sequence group,  
(4) at least five contiguous nucleotides nonidentical to the sequence group,  
(5) at least six contiguous nucleotides nonidentical to the sequence group,  
15 (6) at least seven contiguous nucleotides nonidentical to the sequence group.

50. The isolated nucleic acid molecule of claim 48, wherein the fragment has a size selected from the group consisting of at least: 8 nucleotides, 10 nucleotides, 12 nucleotides, 14 nucleotides, 16 nucleotides, 18 nucleotides, 20, nucleotides, 22 nucleotides, 24  
20 nucleotides, 26 nucleotides, 28 nucleotides, 30 nucleotides, 50 nucleotides, 75 nucleotides, 100 nucleotides, and 200 nucleotides.

51. The isolated nucleic acid molecule of claim 48, wherein the molecule encodes a polypeptide which, or a fragment of which, binds a human HLA receptor or a human  
25 antibody.

52. An expression vector comprising an isolated nucleic acid molecule of any of claims 46-51 operably linked to a promoter.

30 53. An expression vector comprising a nucleic acid operably linked to a promoter, wherein the nucleic acid is a NA Group 2 molecule.

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54. An expression vector comprising a NA Group 1 or Group 2 molecule and a nucleic acid encoding an HLA molecule.

55. A host cell transformed or transfected with an expression vector of claim 52.

5

56. A host cell transformed or transfected with an expression vector of claims 53 or 54.

57. A host cell transformed or transfected with an expression vector of claim 52 and further comprising a nucleic acid encoding HLA.

10

58. A host cell transformed or transfected with an expression vector of claim 53 and further comprising a nucleic acid encoding HLA.

59. An isolated polypeptide encoded by the isolated nucleic acid molecule of claim 46 or claim 47.

15

60. A fragment of the polypeptide of claim 59 which is immunogenic.

61. The fragment of claim 60, wherein the fragment, or a portion of the fragment, binds HLA or a human antibody.

20

62. An isolated fragment of a human cancer associated antigen precursor which, or portion of which, binds HLA or a human antibody, wherein the precursor is encoded by a nucleic acid molecule that is a NA Group 1 molecule.

25

63. The fragment of claim 62, wherein the fragment is part of a complex with HLA.

64. The fragment of claim 63, wherein the fragment is between 8 and 12 amino acids in length.

30

65. An isolated polypeptide comprising a fragment of the polypeptide of claim 59 of sufficient length to represent a sequence unique within the human genome and identifying a

polypeptide that is a human cancer associated antigen precursor.

66. A kit for detecting the presence of the expression of a human cancer associated antigen precursor comprising

5 a pair of isolated nucleic acid molecules each of which consists essentially of a molecule selected from the group consisting of (a) a 12-32 nucleotide contiguous segment of the nucleotide sequence of any of the NA Group 1 molecules and (b) complements of ("a"), wherein the contiguous segments are nonoverlapping.

10 67. The kit of claim 66, wherein the pair of isolated nucleic acid molecules is constructed and arranged to selectively amplify an isolated nucleic acid molecule that is a NA Group 3 molecule.

68. A method for treating a subject with a disorder characterized by expression of a human cancer associated antigen precursor, comprising

15 administering to the subject an amount of an agent, which enriches selectively in the subject the presence of complexes of an HLA molecule and a human cancer associated antigen, effective to ameliorate the disorder, wherein the human cancer associated antigen is a fragment of a human cancer associated antigen precursor encoded by a nucleic acid molecule  
20 selected from the group consisting of

- (a) a nucleic acid molecule comprising NA group 1 nucleic acid molecules,
- (b) a nucleic acid molecule comprising NA group 3 nucleic acid molecules,
- (c) a nucleic acid molecule comprising NA group 5 nucleic acid molecules.

25 69. The method of claim 68, wherein the disorder is characterized by expression of a plurality of human cancer associated antigen precursors and wherein the agent is a plurality of agents, each of which enriches selectively in the subject the presence of complexes of an HLA molecule and a different human cancer associated antigen, wherein at least one of the human cancer associated antigens is encoded by a NA Group 1 molecule.

30 70. The method of claim 69, wherein the plurality is at least 2, at least 3, at least 4, or at least 5 such agents.



71. The method of claims 68-70, wherein the agent is an isolated polypeptide selected from the group consisting of PP Group 1, PP Group 2, PP Group 3, PP Group 4 and PP Group 5 polypeptides.

5 72. The method of claims 68-70, wherein the disorder is cancer.

73. The method of claims 71, wherein the disorder is cancer.

74. A method for treating a subject having a condition characterized by expression of a  
10 human cancer associated antigen precursor in cells of the subject, comprising:  
    (i) removing an immunoreactive cell containing sample from the subject,  
    (ii) contacting the immunoreactive cell containing sample to the host cell under  
conditions favoring production of cytolytic T cells against a human cancer associated antigen  
which is a fragment of the precursor,  
15      (iii) introducing the cytolytic T cells to the subject in an amount effective to lyse  
cells which express the human cancer associated antigen, wherein the host cell is transformed  
or transfected with an expression vector comprising an isolated nucleic acid molecule  
operably linked to a promoter, the isolated nucleic acid molecule being selected from the  
group of nucleic acid molecules consisting of NA Group 1, NA Group 2, NA Group 3, NA  
20 Group 4, and NA Group 5.

75. The method of claim 74, wherein the host cell recombinantly expresses an HLA  
molecule which binds the human cancer associated antigen.

25 76. The method of claim 74, wherein the host cell endogenously expresses an HLA  
molecule which binds the human cancer associated antigen.

77. A method for treating a subject having a condition characterized by expression of a  
human cancer associated antigen precursor in cells of the subject, comprising:

- 30      (i) identifying a nucleic acid molecule expressed by the cells associated with said  
condition, wherein said nucleic acid molecule is a NA Group 1 molecule;  
    (ii) transfecting a host cell with a nucleic acid selected from the group consisting

of (a) the nucleic acid molecule identified, (b) a fragment of the nucleic acid identified which includes a segment coding for a human cancer associated antigen, (c) deletions, substitutions or additions to (a) or (b), and (d) degenerates of (a), (b), or (c);

(iii) culturing said transfected host cells to express the transfected nucleic acid molecule, and;

(iv) introducing an amount of said host cells or an extract thereof to the subject effective to increase an immune response against the cells of the subject associated with the condition.

78. The method of claim 77, further comprising identifying an MHC molecule which presents a portion of an expression product of the nucleic acid molecule, wherein the host cell expresses the same MHC molecule as identified and wherein the host cell presents an MHC binding portion of the expression product of the nucleic acid molecule.

79. The method of claim 77, wherein the immune response comprises a B-cell response or a T cell response.

80. The method of claim 79, wherein the response is a T-cell response which comprises generation of cytolytic T-cells specific for the host cells presenting the portion of the expression product of the nucleic acid molecule or cells of the subject expressing the human cancer associated antigen.

81. The method of claim 77, wherein the nucleic acid molecule is a NA Group 3 molecule.

82. The method of claims 77 or 78, further comprising treating the host cells to render them non-proliferative.

83. A method for treating or diagnosing or monitoring a subject having a condition characterized by expression of an abnormal amount of a protein encoded by a nucleic acid molecule that is a NA Group 1 molecule, comprising

administering to the subject an antibody which specifically binds to the protein or a peptide derived therefrom, the antibody being coupled to a therapeutically useful agent, in an

amount effective to treat the condition.

84. The method of claim 83, wherein the antibody is a monoclonal antibody.

5 85. The method of claim 84, wherein the monoclonal antibody is a chimeric antibody or a humanized antibody.

86. A method for treating a condition characterized by expression in a subject of abnormal amounts of a protein encoded by a nucleic acid molecule that is a NA Group 1 nucleic acid  
10 molecule, comprising  
administering to a subject a pharmaceutical composition of any one of claims 13-25 and 37-45 in an amount effective to prevent, delay the onset of, or inhibit the condition in the subject.

15 87. The method of claim 86, wherein the condition is cancer.

88. The method of claim 86, further comprising first identifying that the subject expresses in a tissue abnormal amounts of the protein.

20 89. The method of claim 87, further comprising first identifying that the subject expresses in a tissue abnormal amounts of the protein.

90. A method for treating a subject having a condition characterized by expression of abnormal amounts of a protein encoded by a nucleic acid molecule that is a NA Group 1  
25 nucleic acid molecule, comprising  
(i) identifying cells from the subject which express abnormal amounts of the protein;  
(ii) isolating a sample of the cells;  
(iii) cultivating the cells, and  
(iv) introducing the cells to the subject in an amount effective to provoke an immune  
30 response against the cells.

91. The method of claim 90, further comprising rendering the cells non-proliferative, prior

to introducing them to the subject.

92. A method for treating a pathological cell condition characterized by aberrant expression of a protein encoded by a nucleic acid molecule that is a NA Group 1 nucleic acid molecule, comprising

administering to a subject in need thereof an effective amount of an agent which inhibits the expression or activity of the protein.

93. The method of claim 92, wherein the agent is an inhibiting antibody which selectively binds to the protein and wherein the antibody is a monoclonal antibody, a chimeric antibody or a humanized antibody.

94. The method of claim 92, wherein the agent is an antisense nucleic acid molecule which selectively binds to the nucleic acid molecule which encodes the protein.

95. The method of claim 92, wherein the nucleic acid molecule is a NA Group 3 nucleic acid molecule.

96. A composition of matter useful in stimulating an immune response to a plurality of a proteins encoded by nucleic acid molecules that are NA Group 1 molecules, comprising a plurality of peptides derived from the amino acid sequences of the proteins, wherein the peptides bind to one or more MHC molecules presented on the surface of the cells which express an abnormal amount of the protein.

97. The composition of matter of claim 96, wherein at least a portion of the plurality of peptides bind to MHC molecules and elicit a cytolytic response thereto.

98. The composition of matter of claim 97, further comprising an adjuvant.

99. The composition of matter of claim 98, wherein said adjuvant is a saponin, GM-CSF, or an interleukin.

100. The composition of matter of claim 96, further comprising at least one peptide useful in stimulating an immune response to at least one protein which is not encoded by nucleic acid molecules that are NA Group 1 molecules, wherein the at least one peptide binds to one or more MHC molecules.

5

101. An isolated antibody which selectively binds to a complex of:

(i) a peptide derived from a protein encoded by a nucleic acid molecule that is a NA Group 1 molecule and

(ii) and an MHC molecule to which binds the peptide to form the complex,

10 wherein the isolated antibody does not bind to (i) or (ii) alone.

102. The antibody of claim 101, wherein the antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody, or a fragment thereof.

15 103. A method for making a nucleic acid or polypeptides encoded thereby comprising culturing the host cell of claim 55 or claim 56 and isolating the nucleic acid or polypeptide from the host cells or culture medium.

20 104. A method for making a nucleic acid or polypeptide encoded thereby comprising providing a non-cell system for transcription and/or translation of a nucleic acid, introducing the nucleic acid of any of claims 46-48 or the expression vector of claim 52 into the non-cell system,  
incubating the non-cell system under conditions sufficient for transcription or translation of the nucleic acid of any of claims 46-48 or the expression vector of claim 52 and  
25 isolating the transcribed nucleic acid or translated polypeptide from the non-cell system.

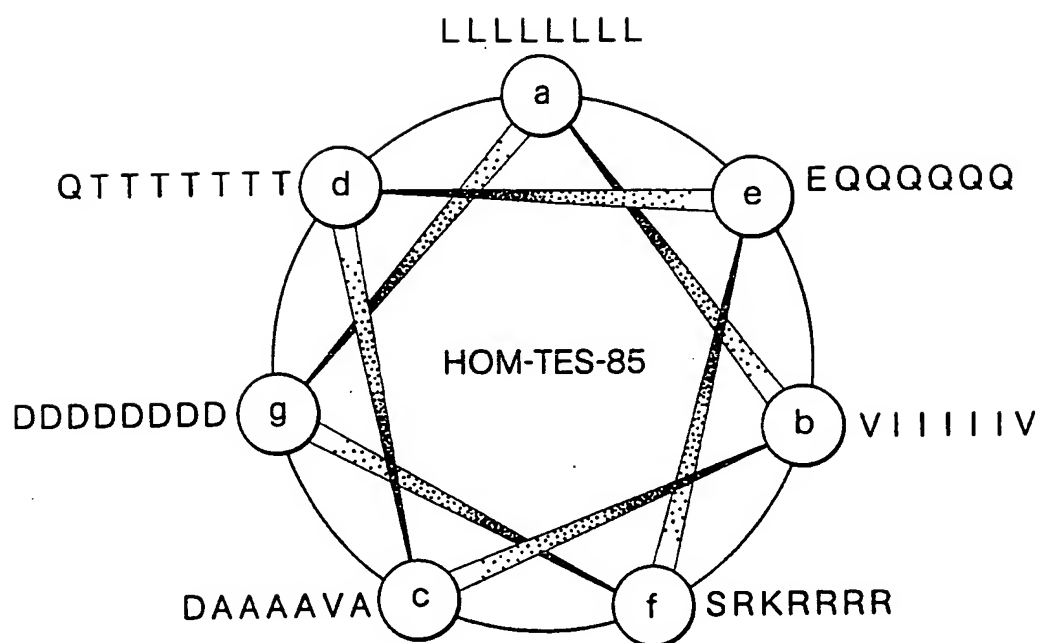


Fig. 1

-1-

## SEQUENCE LISTING

&lt;110&gt; Ludwig Institute for Cancer Research

5 &lt;120&gt; CANCER ASSOCIATED ANTIGENS AND USES THEREFOR

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